OBESITY IN CHRONIC INFLAMMATION

USING RHEUMATOID ARTHRITIS AS A MODEL:

DEFINITION, SIGNIFICANCE, AND EFFECTS OF

PHYSICAL ACTIVITY & LIFESTYLE

A thesis submitted in partial fulfilment of the requirements

of the University of Wolverhampton for the degree of Doctor of Philosophy

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This work or any part thereof has not previously been presented in any form to the University or to any other body whether for the purposes of assessment, publication or for any other purpose (unless otherwise indicated). Save for any express acknowledgments, references and/or bibliographies cited in the work, I confirm that the intellectual content of the work is the result of my own efforts and of no other person.

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Abstract

Background: Inflammation is the natural reaction of the body to an antigen. In some conditions, this reaction continues even after the elimination of the antigen, entering a chronic stage; it targets normal cells of the body and causes extensive damage. Rheumatoid arthritis (RA) is such a condition. It associates with significant metabolic alterations that lead to changes in body composition and especially body fat (BF) increases. In the general population, increased body fat (i.e. obesity) associates with a number of health disorders such as systemic low grade inflammation and a significantly increased risk for cardiovascular disease (CVD). Both effects of obesity could have detrimental effects in RA. Increased inflammation could worsen disease activity while obesity could further increase the already high CVD risk in RA. However, obesity in RA has attracted minimal scientific attention.

Aims: The present project aimed to: 1) assess whether the existing measures of adiposity are able to identify the changes in body composition of RA patients, 2) if necessary develop RA-specific measures of adiposity, 3) investigate the association of obesity with disease characteristics and CVD profile of the patients, 4) and identify factors that might affect body weight and composition in these patients.

Methods: A total of 1167 volunteers were assessed. Of them 43 suffered from osteoarthritis and 82 were healthy controls. These, together with 516 RA patients were used in the first study. Their body mass index (BMI), BF, and disease characteristics were assessed. In the second, third, fourth and fifth studies a separate set of 400 RA patients was assessed. In addition to the above assessments, their cardiovascular profile and more detailed disease

characteristics were obtained. For the final study, 126 RA patients were assessed for all the above and also data on their physical activity levels and their diet were collected.

Results: Assessments of adiposity for the general population are not valid for RA patients. Thus, we proposed RA-specific measures of adiposity. These are able to better identify RA patients with increased BF. We were also able to find associations between obesity and disease activity. Both underweight and obese RA patients had more active disease compared to normal-weight patients. Obese patients had significantly worse CVD profile compared to normal-weight. The newly devised measures of adiposity were able to identify those at increased risk. However, not all obese individuals were unhealthy and not all normal-weight healthy. Among our patients we were able to identify subtypes of obesity with distinct phenotypic characteristics that warrant special attention. Finally, we were able to identify factors that influence body weight and composition. Cigarette smoking protected against obesity while its cessation associated with increased adiposity. Physical activity was also found to be protective against obesity while diet or inflammation of the disease failed to produce any significant results.

Conclusions: Obesity is a significant threat to the health of RA patients. The measures of adiposity developed herein should be used to identify obese RA patients. Physical activity seems like the sole mode for effective weight management in this population. Health and exercise professionals should actively encourage their patients to exercise as much as they can. This study has created more questions than it answered; further research in the association of obesity and inflammation, as well as in ways to treat it, is essential.

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List of Abbreviations

ACR: American college of rheumatology
ACSM: American college of sports medicine
ANCOVA: analysis of co-variance
ANOVA: analysis of variance
Anti-CCP: anti-cyclic citrullinated peptide
AP-1: activator protein one
BF: body fat
BIA: bioelectrical impedance analysis
BMI: body mass index
BP: blood pressure
CHD: coronary heart disease
CI: confidence intervals
CRP: C-reactive protein
CS: current smokers
CVD: cardiovascular disease
DAS: disease activity score
DEXA: dual energy X-ray absorptiometry
DMARDs: disease modifying anti-rheumatic drugs
ESR: erythrocyte sedimentation rate
FFM: fat free mass
HAQ: health assessment questionnaire
HC: healthy controls
HDL: high density lipoprotein
HLA: human leukocyte antigen
HOMA: homeostasis assessment model
IL: interleukin
IPAQ: international physical activity questionnaire
IR: insulin resistance
LCDs: low caloric diets
LDL: low density lipoprotein
LIMAG: limits of agreement
MetS: metabolic syndrome
MHC: major histocompatibility complex
MHO: metabolically healthy but obese
MI: myocardial infraction
MONW: metabolically obese but normal weight
N: number
NF-κB: nuclear factor kappa beta
NS: never smokers
NSAIDs: non-steroidal anti-inflammatory drugs
OA: osteoarthritis
OR: odds ratios
QUICKI: quantitative insulin sensitivity check index
RA: rheumatoid arthritis
REE: resting energy expenditure
RF: rheumatoid factor
RR: relative risk
sd: standard deviation
TDEE: total daily energy expenditure
TEE: thermal effect of exercise
TEF: thermal effect of feeding
Th: T-helper cells
TNFα: tumour necrosis factor alpha
VLCDs: very low caloric diets
WAT: white adipose tissue
WHO: world health organisation
XS: ex-smokers

Responsibilities

The responsibilities of the author in the present project were to:

- Review the available literature and devise the research questions
- Design the methodology in order to address these questions
- Recruit and assess participants
- Manage, input and analyse the data
- Interpret the results
- Disseminate the findings via scientific conferences and peer-reviewed journals
- Submit a thesis to the University of Wolverhampton for the degree of doctor of philosophy

Contributions of collaborative colleagues:

- Advice on the design of the projects
- Patient recruitment
- Edit manuscripts

Dissemination of Findings

Full paper publications in refereed journals

Published

Chapter 2 (parts): Toms T.E., Panoulas V.F., Stavropoulos-Kalinoglou A., *et al.* (2008) "Cardiovascular" Drugs in Rheumatoid Arthritis: Killing Two Birds with One Stone? *IEMAMC.* 8, 259-74

Chapter 5.1: Stavropoulos-Kalinoglou, A., Metsios, G.S., Koutedakis, Y., *et al.* (2007) Redefining overweight and obesity in rheumatoid arthritis patients. *Ann Rheum Dis,* 66, 1316-21.

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Conference presentations

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Chapters 5.1 & 5.2: Stavropoulos-Kalinoglou A., Metsios G.S., Koutedakis Y., *et al.* Redefining Overweight and Obesity in Rheumatoid Arthritis Patients: The Necessity for Disease Specific Measures of Adiposity. The ninth Great British

Research and R&D Show. House of Commons, London, 19 March 2007 (p.302-303).

Chapter 5.6: Stavropoulos-Kalinoglou A., Metsios G.S., Koutedakis Y., *et al.* Associations of physical activity, energy intake and inflammation with body weight and composition in patients with rheumatoid arthritis. Abstract Book-Proceedings: 13th Annual Congress of the European College of Sports Science, 8-13 July 2008, Estoril, Portugal, p. 348, 2008.

Chapter 1: Introduction

Inflammation [from Latin: *inflammatio* i.e. to set on fire (Onions *et al.*, 1996)] is the normal response of the immune system to antigens, such as pathogens, damaged cells, or irritants (Stedman, 2005). It is a complex biological reaction of the vascularised connective tissue that leads to accumulation of fluid and leukocytes in extravascular tissue and can be divided into acute and chronic patterns.

Acute inflammation is the immediate and early response to an infectious agent; it is of short duration, usually lasting minutes, hours or a few days. Since the two major defence components against microbes -_namely antibodies and leukocytes – are normally carried in the bloodstream, acute inflammation has three major components: a) alterations in the vascular calibre that lead to an increase in blood flow, b) structural changes in the microvasculature that permit plasma proteins and leukocytes to leave the circulation, and c) emigration of the leukocytes from the microcirculation and their accumulation in the infected or damaged tissue (Cotran *et al.*, 1999). These alterations are the causes of the five cardinal signs of inflammation as identified by Celsus (30BC-32AD) and R.L.K. Virchow (1821-1902): pain (dolor), heat (calor), redness (rubor), swelling (tumour) and loss of function (functio laesa) (Stedman, 2005).

Acute inflammation may have one of four outcomes: 1) Complete resolution: If the infectious agent is successfully neutralised, inflammation should end with restoration of the site of the inflammation to its normal condition. This is characterised by neutralisation or spontaneous decay of the chemical mediators, normalisation of vascular permeability, cessation of leukocytic infiltration, death of neutrophils, removal of oedema and necrotic debris from the site 2) Abscess

formation: This commonly occurs during infections with pyogenic organisms, such as bacteria. 3) Fibrosis: Healing by connective tissue replacement usually occurs after extensive damage of a tissue, when the inflammatory process takes place in tissue that cannot regenerate, or when there is abundant fibrin exudation. 4) Progression to chronic inflammation: Transition from acute to chronic inflammation occurs when the former cannot be resolved due to the persistence of the infectious agent or to some interference in the process of healing (Cotran *et al.*, 1999).

Chronic inflammation is of longer duration characterised by the presence of lymphocytes and macrophages, proliferation of blood vessels, fibrosis and tissue necrosis in and around the affected tissue. During its course, active inflammation, tissue destruction and repair processes occur simultaneously. Although it may follow acute inflammation, the chronic stage offen begins insidiously, as a low-grade, initially asymptomatic response. This latter type of chronic inflammation is the cause of some common diseases such as atherosclerosis, tuberculosis, lung diseases and rheumatoid arthritis (RA). Chronic inflammation is the result of persistent infections, prolonged exposure to toxic agents, or autoimmunity (Cotran *et al.*, 1999).

Both patterns of inflammation aim to destroy, dilute or fend off the harmful agents but also they initiate the process of healing in the damaged tissues. Inflammation, fundamentally a protective response, is tightly controlled by the immune system as uncontrolled inflammation can be harmful or even fatal. Life-threatening allergies, common chronic inflammatory and autoimmune diseases are such examples (Cotran *et al.*, 1999).

Arthritis (i.e. the inflammation of the joints) is a group of conditions that cause damage to the joints. Several different types of arthritides exist, and they are often categorised as non-inflammatory and inflammatory, mostly due to their pathophysiology. Even though “inflammation” is inherent in the definition of any type of arthritis, this classification is widely used in the medical profession and thus will be used in this thesis as well. Osteoarthritis is the commonest non-inflammatory arthritis; it is most usually caused by mechanical joint damage from age or an injury (Altman *et al.*, 1986). On the other hand, RA is the commonest inflammatory arthritis and will be used in this project as a model for the study of obesity in chronic inflammation. RA is a chronic, progressive, autoimmune, inflammatory disease. It mainly affects synovial joints, producing symmetrical arthritis and is characterised by joint pain and stiffness. If left untreated, it leads to irreversible joint damage and deformity, and ultimately disability (Emery *et al.*, 2002). Even though not fatal *per se*, patients with RA have reduced life expectancy compared to the general population (Erhardt *et al.*, 1989) mainly due to increased prevalence of cardiovascular disease (CVD) (Kitas and Erb, 2003). The exact cause for this remains unknown, however genetic predisposition (Gonzalez-Gay *et al.*, 2007), classical CVD risk factors (Panoulas *et al.*, 2007) and inflammation related to the disease (Gonzalez *et al.*, 2008) all contribute. Interestingly, RA also associates with altered body composition. The chronic inflammation of the disease and especially the activation of the nuclear factor kappa-beta (NF-κβ) pathway (discussed in detail in Chapter 2.1.6.3) trigger the degradation of lean tissue mass and especially muscle mass (Roubenoff *et al.*, 1994). Indeed, RA patients have reduced muscle mass in the presence of unchanged body weight (Roubenoff *et al.*, 1994). In combination with their

sedentary lifestyle, this leads to increased accumulation of body fat (Stavropoulos-Kalinoglou *et al.*, 2007) and could potentially have detrimental effects on both the disease itself and on the overall health of a patient.

Increased adiposity - or obesity - is a well established risk factor for the development of CVD in the general population and is suggested to be the underlying cause of the metabolic syndrome – a constellation of classical CVD risk factors (such as hypertension, hypercholesterolaemia, and hyperinsulinaemia) that results in a two to three-fold increase in CVD risk (Bray and Bellanger, 2006). Moreover, it is now recognised that adipose tissue is not merely an energy storage depot but it is an active endocrine/paracrine organ that secretes a number of bioactive molecules known as adipokines (Mohamed-Ali *et al.*, 1998). Adipokines have several different functions such as regulation of energy intake and expenditure (Houseknecht *et al.*, 1998, Chandran *et al.*, 2003); however, most of them are also implicated in regulation of inflammation (Mohamed-Ali *et al.*, 1998). As a general rule, increased adiposity associates with heightened production of pro-inflammatory molecules, whereas reduced adiposity associates with decreased concentration of such molecules and increased concentration of anti-inflammatory ones (Ramos *et al.*, 2003). The close associations of obesity both with CVD risk and inflammation as well as the body composition changes of the RA patients render the concomitant study of the two conditions highly significant.

In the following pages, the author presents the current knowledge on the aetiology, pathophysiology, epidemiology and treatment of RA. Thereafter, the basic concepts of obesity are discussed, focusing mostly on the functions of adipose tissue as a secretory organ. Also, existing evidence on the associations

of RA with obesity are reviewed. The several questions that arise from this review form the basis of the various studies of the present project. The hypotheses, methods and findings of the studies are presented and discussed in the relevant chapters. Finally, the clinical applications and future recommendations for practice and research are discussed in the general discussion.

Chapter 2: Literature Review

(Parts of this chapter have been published in the journal *Immunology, Endocrine & Metabolic Agents - Medicinal Chemistry* (2008), 8, 259-74)

2.1 Rheumatoid Arthritis

2.1.1 Historical Concepts and Definition

RA was first described in 1800 as a new form of gout under the designation "primary asthenic gout". The author identified several distinctive characteristics of the disease, including predominance in women, a chronic course, involvement of many joints from the onset and a decline in general health (Landré Beauvais, 2001). The term Rheumatoid Arthritis was later introduced to characterise this disease. Rheumatoid (from Greek: *ρεύμα [rhevma]* meaning the flow; and suffix –oid meaning similar to, alike) signifies the chronic cyclic nature of the disease which is characterised by periods of increased activity (flares) and others of remission. Arthritis (from Greek: *άρθρωση [arthrosi]* meaning joint; and suffix –itis meaning inflammation) is literally the inflammation of the joints (Onions *et al.*, 1996). Thus, RA is a systemic chronic inflammatory disorder of cyclic nature that affects mainly synovial joints (Hunder, 2005).

2.1.2 Clinical Manifestations

RA affects any joint where cartilage overlies bone and with a joint cavity lined by a synovial membrane that contains synovial fluid. The changes to the synovium (i.e. oedema, increased vascularity and hyperplasia), the primary site of inflammation in RA, are important to the course of the disease (Buch and Emery, 2002).

RA usually initiates its course of articular symptoms over a period of several weeks to months, however in almost a third of all RA patients disease onset is rapid, occurring over a few days or weeks. At the initial stages of the disease the majority of patients present with oligoarthritis (i.e. less than six joints affected) often asymmetric; monoarthritis is very rare (Hunder, 2005). Stiffness following prolonged periods of rest (i.e. sleep) is also noted as are signs of fatigue.

As the disease progresses the majority of the patients develops polyarthritis and experience unexplained flares and remissions. Even though the course of the disease differs between individuals, most RA patients develop destructive arthritis which can be disabling (Scott *et al.*, 2000). Among patients with long-standing disease (i.e. >10 years) only 17% are free from any disability while 16% are completely disabled (Sherrer *et al.*, 1986). As early as 1949 (Steinbrocker *et al.*), this difference in disease outcomes was known and a classification of the functional limitations of the disease was proposed (Table 1). This classification is important in the planning of future health needs and treatment. More worryingly though, survival rates of RA patients are significantly lower compared to that of controls (Wolfe *et al.*, 1994). All these have a significant impact both on the patient and their families as well as the society in general as the costs incurred by RA are significant (Cooper, 2000).

Table 1: Functional classification of Rheumatoid Arthritis (adopted from Steinbrocker *et al.*, 1949)

Class	Description
I	No limitations
II	Function is adequate for normal activities despite joint discomfort or limitation of motion
III	Function is inadequate for most self care and vocational activities
IV	Patient is largely or wholly unable to manage self-care and may be restricted to a wheelchair or bed

2.1.3 Diagnosis and Treatment

Early and effective interventions can minimise the destructive course of RA (O'Dell, 2002), thus prompt identification of individuals with RA is essential. Unfortunately, the differential diagnosis of polyarthritis is extensive and includes conditions such as inflammatory bowel disease, psoriatic arthritis, acute rheumatic fever, human-immunodeficiency-virus infection, gout, hyper- and hypothyroidism, systemic lupus erythematosus, osteoarthritis, malignancy and several other. Thus a careful examination of patients history, persistence of symptoms, laboratory and radiographic features are essential to establish an accurate diagnosis (Hunder, 2005). In 1987, the American Rheumatism Association (now known as American College of Rheumatology - ARC) devised a number of criteria for the identification of patients with RA. The full list of these criteria is presented in Table 2. At least four of the seven criteria have to be present for a patient to be diagnosed with RA (criteria 1-4 for at least six weeks). These criteria can distinguish RA from other forms of arthritis with a specificity of 89% and a sensitivity of 94% (Arnett *et al.*, 1988).

Table 2: The 1987 American College of Rheumatology revised criteria for the classification of rheumatoid arthritis (adopted form Arnett *et al.*, 1988)

Criterion	Description
1. Morning Stiffness	Morning stiffness in and around the joints, lasting at least one hour before maximal improvement
2. Arthritis of 3 or more joint areas	At least three joint areas (out of 14 possible; right or left proximal interphalangeal , metacarpophalangeal, wrist, elbow, ankle, metatarsophalangeal joints) simultaneously have had soft tissue swelling or fluid (not bony overgrowth alone) as observed by a physician
3. Arthritis of hand joints	At least one area swollen (as defined in criterion two) in a wrist, metacarpophalangeal, or proximal interphalangeal joint
4. Symmetric arthritis	Simultaneous involvement (as in criterion two) of the same joint areas on both sides of the body (bilateral involvement of interphalangeal, metacarpophalangeal, or metatarso-phalangeal joints without absolute symmetry is acceptable)
5. Rheumatoid nodules	Subcutaneous nodules over bony prominences or extensor surfaces, or in juxta-articular regions as observed by a physician
6. Serum rheumatoid factor	Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in <5% of normal control subjects
7. Radiographic changes	Radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal body decalcification localised in, or most marked adjacent to, the involved joints (osteoarthritis changes alone do not qualify)

Unfortunately, to date, there is no known cure for RA. However, several different medical approaches are used to treat it. Their main aims are to relieve symptoms (i.e. joint pain, swelling and stiffness) or to decelerate the progression of joint damage. The choice of medication depends on the severity of the disease and its symptoms as well as the response of the patient to them. The most commonly used medicines are described below.

Analgesics: More commonly known as painkillers, they aim at pain relief rather than reduction of inflammation. The most commonly prescribed analgesic is paracetamol. Codeine is another analgesic, which is sometimes prescribed as a combined medicine with paracetamol. This is known as co-codamol.

Non-steroidal anti-inflammatory drugs (NSAIDs): Several different types of NSAIDs exist. Ibuprofen and aspirin are those most commonly used. Diclofenac, fenoprofen and flurbiprofen are also frequently prescribed. A new type of NSAIDs, called COX-2 (cyclooxigenase-2) inhibitors is also available. Like analgesics, NSAIDs help to relieve pain while they can also reduce stiffness and inflammation. However, they don't affect disease progression. They act by blocking the action of the enzyme cyclo-oxygenase. This enzyme, and especially its second form (i.e. COX-2), produces prostaglandins which are involved in the inflammatory cascade of RA (McWhorter, 1988). When taken in high doses, or over a long period of time, NSAIDs can cause complications, such as digestive problems, stomach bleeding, kidney and liver damage, tinnitus (ringing in the ears) and high blood pressure. COX-2 inhibitors are generally less harmful to the stomach, however they might increase CVD risk (Mukherjee *et al.*, 2001).

Corticosteroids: Drugs such as prednisone and methylprednisolone are used to reduce pain and inflammation and can also reduce joint damage (Pisetsky and St Clair, 2001). They are usually used when NSAIDs fail to provide relief (Verhoeven *et al.*, 1998). They are prescribed on a short term basis, most often during a flare-up. Relief is rapid and the effect can last from a few weeks to several months depending on the severity of the disease. Such drugs act directly on the immune system and lower its response to the antigen (Vane and Botting, 1987). However, this has a significant impact on the ability of the body to fend off

harmful agents such as viruses and bacteria (Doran *et al.*, 2002a). Other side-effects of corticosteroids include weight gain, osteoporosis, easy bruising, muscle weakness, and thinning of the skin. They can also worsen diabetes and glaucoma and increase risk for CVD (Panoulas *et al.*, 2008b).

Disease modifying anti-rheumatic drugs (DMARDs): DMARDs are the second line of defence against RA. They were initially prescribed when the above options failed to produce sufficient results; nowadays however they are used earlier and earlier in the course of the disease. While NSAIDs focus on reduction of symptoms, DMARDs aim to reduce the destructive effect of inflammation on the joints. A drawback of these drugs is that it may take several months before their action is noticed, thus early intervention is imperative. Depending on the specific medicine the mechanism of action differs; however, all focus on limitation of the damage caused by inflammation to the bones, tendons, ligaments and cartilage of the joint (Kremer, 2001). The most commonly prescribed DMARDs at present include methotrexate, sulfasalazine, hydroxychloroquine and leflunomide and can be prescribed either alone or in combination with each other; older drugs, such as gold injections and penicillamine are now rarely used.

Biologics: Tumour necrosis factor alpha (TNFα) blockers are a more recent type of DMARD that act faster compared to other DMARDs. The most commonly prescribed such medications include infliximab, etanercept and adalimumab. TNFα blockers can usually reduce symptoms within a few weeks and can also slow down or even halt the progression of RA. They bind TNFα in the joint as well as the circulation and prevent its interaction with the cells it targets. This leads to significant reductions in circulating TNFα and most importantly to rapid relief of symptoms as well as minimising the effects of RA on the joints (Louie *et al.*,

2003). However, TNFα blockers may have serious side-effects. They can cause heart failure, infection and lymphoma amongst others (Fleischmann *et al.*, 2004). Interleukin (IL)-1 and -6 blockers are also available. Anakinra blocks the biologic activity of naturally occurring IL-1, improving inflammation and cartilage degradation associated with RA, by competitively inhibiting the binding of IL-1 to the IL-1 type receptor. Even though it is safer compared to TNFα blockers, it is not as effective in preventing joint damage (Fleishmann, 2002). MRA is an anti-IL-6 receptor monoclonal antibody that acts by blocking the activity of IL-6. It has been shown to effectively reduce inflammation and minimise joint damage in RA patients. However, it is a very new drug and its long-term side effects have not yet been extensively studied (Choy *et al.*, 2002, Nishimoto *et al.*, 2004).

Surgery: In severe cases of RA, arthroplasty (reconstruction or replacement of the affected joint) or osteotomy (shortening or lengthening of the bone) is sometimes required. Its main aim is to restore movement and function of the joint (Coventry, 1973). Most commonly operated joints are the hips and knees. In the hands most surgeries aim to repair damaged tendons (Clayton, 1965).

2.1.4 Epidemiology

Several studies in various countries have investigated the prevalence and incidence of RA and a considerable variation between different populations has been found (Table 3). However, in Northern Europe and North America a prevalence of 0.5-1% with an annual incidence of 20-50 new cases per 100.000 inhabitants is usually reported (Alamanos and Drosos, 2005). These values seem to have decreased compared to older ones (Guillemin *et al.*, 1994, Shichikawa *et al.*, 1999, Doran *et al.*, 2002b) however it is not clear whether this is a true reduction or merely a reflection of different methods used to identify RA patients (Alamanos and Drosos, 2005).

RA has also been shown to reduce life expectancy by 3-10 years depending on severity of the disease and age at onset (Erhardt *et al.*, 1989). The increases in life expectancy recorded in the general population over the past decades are not reflected in RA patients (Glennas *et al.*, 2000, Buch and Emery, 2002). The causes of death do not differ significantly between RA patients and the general population, but they occur at a younger age (Alamanos and Drosos, 2005). The main cause of death in RA is CVD which is both more prevalent and more likely to lead to death in these patients than in the general population (Glennas *et al.*, 2000, Kitas and Erb, 2003).

Table 3: Prevalence and incidence of Rheumatoid Arthritis in different countries (adopted form Alamanos and Drosos, 2005)

Population		Prevalence rates	Incidence rates
North America	USA (general population)	0.9–1.1	0.02–0.07
	USA (native-Americans)	5.3–6.0	0.09–0.89
North Europe	England	0.8–1.10	0.02–0.04
	Finland	0.8	0.03–0.04
	Sweden	0.5–0.9	
	Norway	0.4–0.5	0.02–0.03
	Netherlands	0.9	0.05
	Denmark	0.9	
	Ireland	0.5	
South Europe	Spain	0.5	
	France	0.6	0.01
	Italy	0.3	
	Greece	0.3–0.7	0.02
	Yugoslavia	0.2	
South America	Argentina	0.2	
	Brazil	0.5	
	Colombia	0.1	
Asia	Japan	0.3	0.04–0.09
	China	0.2–0.3	
	Taiwan		0.3
	Indonesia	0.2–0.3	
	Philippines	0.2	
	Pakistan	0.1	
Middle East	Egypt	0.2	
	Israel	0.3	
	Oman	0.4	
	Turkey	0.5	
Africa		0–0.3	

2.1.5 Aetiology and Risk Factors

The aetiology of RA is not clear. It is believed that RA is triggered when an immunogenetically susceptible host is exposed to an antigen. In this manner, an acute inflammatory reaction is initiated. In contrast to the normal course of inflammation, when the antigen has been repelled, this reaction is not terminated. Instead, it recognises some of the tissues in the joints as foreign and attacks them (autoimmune reaction). It is this chronic uncontrolled phase of the inflammation that ultimately destroys the joints. The antigen(s) that triggers the initial inflammation has not been positively recognised; however, several potential risk factors have been identified and are discussed below.

2.1.5.1 Genetic Susceptibility

Studies have found high rates of concordance between monozygotic twins (Jarvinen and Aho, 1994) and first-degree relatives of RA patients (Cotran *et al.*, 1999) suggesting a definite genetic predisposition to RA. However, the failure to demonstrate Mendelian inheritance patterns indicates interplay of multiple genetic factors (Ollier and MacGregor, 1995, Buch and Emery, 2002).

The most consistent genetic association of RA is with the human leukocyte antigen (HLA) alleles (Cotran *et al.*, 1999, Buch and Emery, 2002). HLA is a part of the major histocompatibility complex (MHC) of genes, located in chromosome six. Its main function is to encode molecules (Class I and II) responsible for presenting T-cells (a lymphocyte sub-type of the immune system capable of destroying virally infected cells) with viral or other peptides. The majority of individuals who develop RA (65%-80%) have the HLA-DR4 or DR1 allele or both. All the DR alleles associated with RA share a common region of four amino acids

located in the antigen-binding cleft of the DR molecule adjacent to the T-cell receptor (Cotran *et al.*, 1999). This is referred to as “the shared” or “the rheumatoid epitope” (Alamanos and Drosos, 2005) and is presumably the specific binding site of the antigen that initiates the inflammation of the joints (Cotran *et al.*, 1999). Differences in genes of various proteins implicated in the course of RA (i.e. cytokines) have also been studied; however, their results were not consistent between different populations (Hajeer *et al.*, 2000, Martinez *et al.*, 2000). This indicates that, in addition to HLA, the development and progression of RA is influenced by a complex genetic profile.

2.1.5.2 Age and Gender

Even though RA is not age or gender specific, differences between age groups and genders exist. Most epidemiological studies suggest an age of disease onset during or after the fifth decade of life (Guillemin *et al.*, 1994, Shichikawa *et al.*, 1999, Alamanos and Drosos, 2005). However, no age is immune to RA as even young children can suffer from it (Hunder, 2005). Females are two-three times more likely to develop RA compared to males (Gabriel *et al.*, 1999, Symmons *et al.*, 2002, Alamanos and Drosos, 2005).

2.1.5.3 Hormones

The above mentioned gender difference as well as the observations that pregnancy has an ameliorating effect on RA and that RA patients are more likely to be nulliparous before disease onset compared to the general population suggest an influence of hormonal factors in the occurrence and progression of the disease (Hazes, 1991, Buch and Emery, 2002). Overall, androgens have an immunosuppressive role whereas oestrogens are known to stimulate the immune

system. In pregnant women corticotrophin-releasing hormone directly stimulates production of dehydroepiandrosterone, the major androgen in women, by foetal adrenal cells (Smith *et al.*, 1998) and could be the reason for disease remission during pregnancy. In a similar way, oral contraceptives reduce disease severity (van Zeben *et al.*, 1990) or could even protect against its development (Hazes and van Zeben, 1991, Koepsell *et al.*, 1994).

Moreover, during pregnancy, alloantibodies developed against the paternal HLA are found in maternal circulation (Combe *et al.*, 1985). These alloantibodies block the function of HLA-DR epitopes and could thus down-regulate the disease (Moynier *et al.*, 1987, Kim *et al.*, 2003). However, the positive effects of pregnancy on RA revert in the post-partum period (Alamanos and Drosos, 2005).

2.1.5.4 Infectious Agents

Several different infectious agents (e.g. retroviruses, parvoviruses, mycobacteria, Borrelia, Mycoplasma) have been studied as possible “initiators” of RA, however Epstein-Barr virus is currently the most investigated such agent (Krause *et al.*, 1996, Schaeverbeke *et al.*, 1997, Hunder, 2005). This virus shares some homologous HLA-DRβ chain epitopes with type-2 collagen. Joint cartilage is rich in this type of collagen and autoimmunity to it can be demonstrated in most RA patients. It is thus suggested that initial infection with Epstein-Barr virus causes a normal immunological reaction. Due to the similarities of the virus with type-2 collagen, the reaction crosses over to affect joint cartilage (Cotran *et al.*, 1999).

A similar hypothesis exists for *Mycobacterium tuberculosis.* This bacterium produces a number of heat shock proteins (proteins produced by cells of all species in response to stress) which have up to 65% sequence homology with human heat shock proteins (Kaufmann, 1990). It is suggested that antibodies and

T-cells recognise epitopes shared by such proteins of both the infectious agent and the host, facilitating cross-reactivity and triggering an autoimmune response (Buch and Emery, 2002).

2.1.5.5 Other Risk Factors

Smoking has also been implicated in the initiation of the disease. Its effects appear to be dose depended and heavy smoking associates with increased risk for seropositive (i.e. abnormal rheumatoid factor) disease (Wilson and Goldsmith, 1999). Furthermore, smoking has been suggested to affect severity and outcome of RA (Harrison, 2002, Manfredsdottir *et al.*, 2006) but findings in other studies are not consistent (Finckh *et al.*, 2007).

Diet is yet another potential risk factor for the development of RA. Recent studies have indicated that consumption of fish, olive oil and vegetables could protect against initiation of RA (Cleland *et al.*, 2003). Similarly, the increased intake of such food and a concomitant reduction in fat intake can decrease disease severity (Cleland and James, 2002). The protective role of fish and vegetable consumption has been attributed to the effect of omega-3 long chain polyunsaturated fatty acids and other anti-oxidants against the oxidative stress associated with rheumatoid arthritis (Alamanos and Drosos, 2005).

Finally, ethnicity can affect the development of RA. Genetic variations in the rheumatoid epitope and its associations with the progression of the disease have been found in different populations (Drosos *et al.*, 1992, Drosos and Moutsopoulos, 1995, Gorman *et al.*, 2004). This variation in combination with environmental and lifestyle factors (e.g. diet) are most probably responsible for the geographic variation of the disease (Alamanos and Drosos, 2005).

2.1.6 Pathogenesis

Even though our understanding of the aetiology of RA is limited, the pathogenesis of this disease is much clearer. As described previously, an antigen with characteristics similar to a normal cell in the joint triggers an autoimmune reaction which is responsible for the chronic destructive nature of RA.

MHC class II molecules, carrying the antigen, cause naive T cells to divide and differentiate. Activated T cells release a number of bioactive molecules called cytokines. Angiogenic cytokines, such as vascular endothelial growth factor, are responsible for the growth of new blood vessels (Colville-Nash and Scott, 1992, Fearon *et al.*, 2003) as well as rendering them hyper-permeable (Ferrara *et al.*, 1991, Malemud, 2007). The adhesion of leukocytes (i.e. T cells; mainly CD4+ helper T cells, B-cells, macrophages) to the endothelium is initially controlled by E-selectin, an adhesion molecule that forms low-affinity bonds with the leukocytes and causes them to slow down and roll along the blood vessel wall (Ospelt and Gay, 2008). Migration of these cells to the site of inflammation is then facilitated by other adhesion molecules, such as intercellular adhesion molecules and vascular cell adhesion molecules (Malik and Lo, 1996). Cytokines, such as TNFα, IL-1, IL-6, and IL-8, are again implicated in this process by inducing the expression of adhesion molecules on endothelial cells and leukocytes (Mojcik and Shevach, 1997, Nassonov *et al.*, 2000). The main noticeable structural changes are oedema, increased vascularity and hyperplasia of the synovium which manifest clinically as joint swelling and pain (Buch and Emery, 2002).

As the disease progresses, neovascularisation and migration of leukocytes in the synovium continues; hyperplasia of the synovium becomes even more apparent (Henderson *et al.*, 1988). Ultimately, the hypertrophied synovium

becomes invasive at the site of the joint where the synovium attaches to the bone or cartilage. This causes the formation of a distinctive tissue, called “pannus” where a large concentration of matrix metalloproteinases is observed (Ospelt and Gay, 2008). These molecules are involved in the destruction of extracellular matrix and are responsible for the joint erosions observed in RA (Buch and Emery, 2002, Ospelt and Gay, 2008). Cytokines, especially TNFα and IL-1 are known to induce their production.

2.1.6.1 Cytokines

The pathogenesis of RA as described above is a complicated procedure involving several different mechanisms. However, in every step of it the involvement of cytokines is apparent. Cytokines are a category of soluble proteins that serve as chemical messengers between cells. They are produced *de novo* in response to an immune stimulus by a wide variety of cells and can have autocrine, paracrine and endocrine effects. Different cell types can produce the same cytokine or a single cytokine can act on several different cells (i.e. pleiotropy). Several different cytokines can have a similar effect (i.e. redundancy) and they can be produced in a cascade (i.e. stimulate their target cell to produce more cytokines). Finally, cytokines can act synergistically (together with each other) or antagonistically (against each other) (Ashman and Papadimitriou, 1995). Cytokines are involved in several processes including cell growth and differentiation, inflammation, tissue repair and remodelling, and are thus critical to the development and functioning of both the innate and humoral immune responses (Buch and Emery, 2002).

In the rheumatoid synovium the cytokines most often encountered are TNFα, IL-1 and IL-6. Synergistically, TNFα and IL-1 promote a pro-inflammatory profile which induces the functions previously described, namely: T- and B-cell

recruitment and activation, angiogenesis, chemotaxis, vessel permeability and matrix metalloproteinase production (Klimiuk *et al.*, 1997). TNFα is responsible to a greater extent for the proliferative and inflammatory aspects of the disease, whereas IL-1 is for its destructive aspects (Buch and Emery, 2002). IL-6 seems to be implicated more in the systemic effects of RA (discussed in the following section). The sources, functions and effects of cytokines implicated in RA are presented in Table 4.

Table 4: Sources, functions and effects of selected cytokines

Cytokine	Producing Cell	Target Cell	Function	Main Effects
IL-1	monocytes macrophages B cells dendritic cells	Th cells	co-stimulation	damage to proteoglycans in the cartilage
		B cells	maturation and proliferation	
		natural killer cells	activation	
		various	inflammation, acute phase response, fever	
IL-6	monocytes macrophages Th2 cells stromal cells	activated B cells	differentiation into plasma cells	production of acute phase reactants
		plasma cells	antibody secretion	
		stem cells	differentiation	
		various	acute phase response	
TNFa	macrophages mast cells natural killer cells	macrophages	adhesion molecules and cytokine expression	bone and cartilage resorption MHC class II expression chemotaxis increase production of IL-1 and IL-6 activation of adhesion molecules activation of T and B cells muscle degradation
		tumour cells	cell death	

IL: interleukin; TNF: Tumour Necrosis Factor. Th: T helper

2.1.6.2 Systemic Effects of Cytokines

The effects of cytokines are not limited within the synovium. The local inflammation of RA soon triggers a systemic response of the innate immune system (i.e. antigen-nonspecific defence mechanisms that a host is born with), the acute phase response. This response provides an early defence and enables the body to recognize foreign substances early in the infection process prior to the full activation and implementation of the immune responses (Baumann and Gauldie, 1994). It is characterized by leukocytosis, fever, alterations in the metabolism of many organs as well as changes in the plasma concentrations of various acute-phase proteins (Gabay and Kushner, 1999).

Blood-borne cytokines, and especially IL-6 (Smith and McDonald, 1992), that leave the synovium reach the liver and stimulate hepatocytes to synthesize and secrete acute phase proteins; i.e. soluble pattern-recognition receptors that bind onto the pathogen and present it to the respective leukocytes (Cotran *et al.*, 1999). Acute phase proteins have been defined as any protein whose plasma concentrations increases or decreases by at least 25% during an acute inflammatory disorder (Morley and Kushner, 1982). Acute phase response is normally terminated when the pathogen is eliminated. Mechanisms, involving production of anti-inflammatory cytokines (e.g. IL-10) by Kupffer cells (fixed macrophages located in the liver), suppress the expression of IL-6 in the liver and terminate the inflammatory cascade (Suffredini *et al.*, 1999). However, in chronic or recurring inflammation acute phase response is constantly activated increasing significantly the levels of acute phase proteins in the blood.

An important acute phase protein, for RA, is C-reactive protein (CRP). Its levels, normally below 1mg/L of whole blood, can rise 10.000-fold following

infection within very short periods of time (Pepys and Hirschfield, 2003). CRP is a prominent indicator of disease activity in RA as well as other conditions. Increased levels of it indicate uncontrolled disease or a flare while reduced levels indicate disease remission (Crockson *et al.*, 1978). However, most RA patients constantly exhibit CRP levels well above the "accepted" values (i.e. <8mg/L) (Kushner, 1991). Apart from its association with disease activity, CRP has been identified as an independent risk factor for the development of CVD (Ridker *et al.*, 2002, de Ferranti and Rifai, 2007), and inflammation overall is thought to affect the function and health of several different organs and tissues in the body.

2.1.6.3 Transcription Factors

The way cytokines influence all these functions is by affecting gene transcription. In the process of transcription, RNA is produced from the DNA and this conversion is an essential element in gene expression. The central role of transcription in the process of gene expression means that it regulates the expression of genes in particular cell types or in response to a particular stimuli, such as cytokines (Okamoto *et al.*, 2008). To date two such factors have been implicated in the pathogenesis of RA; the nuclear factor kappa beta (NF-κB) and the activator protein one (AP-1).

NF-κB

NF-κB proteins are a family of ubiquitously expressed transcription factors that play an essential role in most immune and inflammatory responses. The NF-κB proteins are retained in an inactive form in the cytoplasm through their interaction with inhibitor of NF-κB proteins (Okamoto *et al.*, 2008). Stimulation by cytokines, such as TNFα and IL-1, phosphorylates the inhibitor proteins and leads to their

ubiquitination and subsequent proteosomal degradation. This enables NF-κB to translocate to the nucleus and stimulate the transcription of genes. Among the numerous genes that NF-κB influences are those of cytokines (TNFα, IL-1, IL-6), adhesion molecules, matrix metalloproteinases and others that control apoptosis and cell proliferation (Makarov, 2001). The NF-κB proteins are highly expressed and activated in the RA synovium (Miagkov *et al.*, 1998).

The increased cellularity of the synovium is a result of the increased recruitment of leukocytes. However, it is also mediated by deficient or even impaired apoptosis resulting from the up-regulation of anti-apoptotic molecules by NF-κB (Perlman *et al.*, 2001, Schedel *et al.*, 2002). Apart from this association with the inhibition of programmed cell death, NF-kB also has an important role in the development and homeostasis of the immune, hepatic, and nervous systems (Okamoto *et al.*, 2008).

AP-1

AP-1 transduces extracellular signals to immune cells, resulting in changes in the expression of specific target genes with an AP-1 binding sites in their promoter or enhancer regions. Even though the knowledge around this transcription factor is relatively limited, several mechanisms by which it may affect the severity of inflammation have been proposed. AP-1 has been suggested to activate cytokine production (in co-operation with other transcription factors), regulate differentiation of naive T cells into Th-1 or Th-2 cells or interact and suppress the glucocorticoid receptors (Okamoto *et al.*, 2008).

2.1.7 Extra-articular Manifestations

A common feature of RA is the presence of extra-articular manifestations with almost 15% of RA patients suffering from such conditions (excluding CVD) (Turesson and Jacobsson, 2004). Extra-articular manifestations usually present in patients with high disease activity and increased markers of inflammation, such as CRP (Turesson *et al.*, 2003) and associate with increased morbidity and mortality (Turesson *et al.*, 1999). All the known extra-articular manifestations are listed in Table 5, however only those relevant to this project are discussed below.

Table 5: Systemic Manifestations of Rheumatoid Arthritis

Organ System	Systemic Manifestation
Cardiovascular	Coronary artery disease, myocardial infraction, pericarditis
Muscle	Rheumatoid cachexia
Blood	Anaemia, thrombocytosis, Felty's syndrome, large granulocytic leukaemia, non-Hodgkin's lymphoma
Pulmonary	Pleural effusion, nodules, interstitial lung disease
Vascular	Vasculitis
Nervous	Neuropathy
Bone	Osteopenia and osteoporosis
Ocular	Keratoconjunctivitis sicca, scleritis, episcleritis, peripheral ulcerative keratitis
Salivary glands	Secondary Sjögren's syndrome with dry eyes and mouth
Skin	Cutaneous vasculitis, nodules

2.1.7.1 Cardiovascular Disease

RA is associated with an estimated 70% excess mortality rate (Pham *et al.*, 2006) that can be ascribed to cardiovascular causes in up to 50% of cases (del Rincon *et al.*, 2001). In this population a 3-fold increased adjusted risk of myocardial infraction has been reported, particularly in subjects with long-standing disease (Solomon *et al.*, 2003). The exact causes for that remain unclear; however, classical CVD risk factors (i.e. hypertension, dyslipidaemia, insulin resistance), the

inflammation related to RA and antirheumatic medication have been shown to affect cardiac health of these patients.

Hypertension is highly prevalent in RA but often remains undiagnosed (Panoulas *et al.*, 2007). Genetic factors, inflammation and commonly used therapies are known to affect it (Panoulas *et al.*, 2007, Panoulas *et al.*, 2008a, Panoulas *et al.*, 2008b, Panoulas *et al.*, 2008c). Dyslipidaemia is also common in RA and associates with disease activity; it manifests as low total and high density lipoprotein (HDL), in the presence of high triglyceride levels (Quyyumi, 2006). This pattern produces a more dense, easily oxidisable, and intensely atherogenic low-density lipoprotein particle (Sattar *et al.*, 2003). Hyperinsulinaemia and insulin resistance are also observed in RA (Paolisso *et al.*, 1991). The potential mechanisms again include use of glucocorticoids but also the direct effects of cytokines, such as TNFα, on impeding insulin-mediated glucose uptake in skeletal muscle and on lipolysis (Sattar *et al.*, 2003).

Inflammation however, is currently considered to be the unifying mechanism that explains cardiovascular risk in RA (Quyyumi, 2006). Atherosclerosis is a chronic inflammatory process of the vascular bed mediated by mononuclear cell infiltration fibrosis, elaboration of cytokines, increased cellular adhesion, and plaque destabilization. This leads to a low-grade systemic inflammatory response that is an indicator of increased future risk of atherothrombotic events (Ross, 1999). In RA, inflammation of the synovium leads to a far greater elevation of circulating cytokines (i.e. IL-1, IL-6 and TNFα) and markers of inflammation (i.e. CRP). Elevated levels of circulating cytokines do not simply reflect increased risk, but, through their pleiotropic effects, directly exacerbate vascular disease (Sattar *et al.*, 2003). As indicated previously,

cytokines can affect glucose handling in the skeletal muscle, and lipid metabolism in the adipose tissue leading to insulin resistance and dyslipidaemia respectively. They also stimulate production of plasminogen activator inhibitor-1 and fibrinogen by the liver, enhancing a prothrombotic state and activating the vascular endothelium (Quyyumi, 2006). The latter has been shown to increase arterial stiffness (a measure of subclinical vascular disease that predicts future CVD) and lead to endothelial dysfunction (the first step in the process of atherosclerosis)(Yasmin *et al.*, 2004). Indeed, RA patients exhibit increased arterial stiffness (Maki-Petaja *et al.*, 2006) and endothelial dysfunction (Hurlimann *et al.*, 2002, Raza *et al.*, 2006) compared to controls.

Control of inflammation with medication can have both beneficial and deleterious effects on CVD risk of RA patients. Corticosteroids exacerbate dyslipidaemia, blood pressure and insulin resistance (del Rincon *et al.*, 2004, Quyyumi, 2006, Panoulas *et al.*, 2008b); despite reducing inflammation, patients treated with high doses of them have greater incidence of carotid plaque and arterial stiffness (del Rincon *et al.*, 2004). Similarly, nonsteroidal-antiinflammatory-agents may exacerbate hypertension, and COX-2 antagonists are associated with increased risk of myocardial infraction (Quyyumi, 2006). In contrast, DMARDs may be protective against cardiovascular risk by reducing risk for myocardial infraction by almost 70% (Choi *et al.*, 2002). Finally, anti-TNFα treatment has been shown to improve arterial stiffness and endothelial function (Maki-Petaja *et al.*, 2006). However, whether this translates into true reduction in cardiovascular risk remains unknown (Quyyumi, 2006) although early results appear promising (Dixon *et al.*, 2007).

2.1.7.2 Rheumatoid Cachexia

RA also has a significant impact on body composition. Almost 2/3 of RA patients experience involuntary wasting of muscle mass accompanied by stable or slightly decreased total body weight (Roubenoff *et al.*, 1994). This condition is termed "rheumatoid cachexia". It differs from the age-related sarcopenia, a qualitative and quantitative decline in skeletal muscle mass, as muscle wasting in rheumatoid arthritis occurs at a younger age and at an accelerated rate (Roubenoff *et al.*, 1994, Walsmith and Roubenoff, 2002). It also differs from cachexia, a condition of rapid muscle degradation accompanied by rapid weight loss, due to the small changes in body weight of RA patients (Rall and Roubenoff, 2004). Thus, rheumatoid cachexia is a distinct condition observed solely in RA.

The exact mechanisms underlying the development of rheumatoid cachexia are not yet clear. However, inflammation of the disease as well as the inactive lifestyle of most RA patients are thought to play a central role in its development (Metsios *et al.*, 2006). TNFα, initially named "cachectin", has been recognised as a potent enhancer of muscle degradation and in conjunction with IL-1 they have been shown to enhance proteinolysis (Zamir *et al.*, 1992). They activate the NF-κB transcription pathway which in turn acts on proteinolytic genes and increases conjugation of ubiquitin (a regulatory protein that labels other proteins for proteosomal degradation) to muscle proteins (Lecker *et al.*, 1999). These proteins are then catalysed to small peptides and disposed by the body (Kisselev *et al.*, 1998). TNFα also reduces insulin action in the muscle (Hotamisligil *et al.*, 1994); this results in limited transportation through muscle-cell membrane of amino acids essential for proteinosynthesis (Rall and Roubenoff, 2004). Control of inflammation with medication has been shown to reduce muscle

catabolism; however it can not reverse rheumatoid cachexia on its own (Metsios *et al.*, 2007).

In order for the above negative effects of inflammation to be reversed, adequate stimuli for muscle growth need to be present. Physical activity, even at very low intensity, is the best such stimulus. Low-intensity aerobic exercise is known to acutely and chronically improve insulin action on the muscle allowing for greater amino-acid delivery which can then be used to synthesise protein (Fujita *et al.*, 2007). Resistance exercise initiates muscle reconstruction mechanisms, mainly through activation of the "mammalian target of rapamycin" signalling pathway (Kubica *et al.*, 2005). Contrary to common belief, RA patients are able to exercise at the required intensities without aggravating their disease and to elicit beneficial changes in their body composition; however very few of them chose to do so (Metsios *et al.*, 2008c). In order for the exercise-induced muscle synthesis to occur, diet needs to provide adequate supply of amino-acids (Hebuterne *et al.*, 2001). However, diet on its own is not enough to reverse rheumatoid cachexia (Marcora *et al.*, 2005a).

The loss of muscle mass in RA patients results in movement limitations and is partly responsible for the functional disability of these patients (Munro and Capell, 1997, Westhovens *et al.*, 1997). Rheumatoid cachexia may also be implicated in the cardiovascular risk of RA patients. As noted previously, muscle wasting is accompanied by very little change in total body weight; this indicates increased quantity of body fat (BF) (Stavropoulos-Kalinoglou *et al.*, 2007). In the general population, increased BF has been proposed as the underlying cause of classical risk factors as well as the metabolic syndrome (a constellation of risk factors and central adiposity which increases the risk for CVD 2-3 fold) (Grundy *et*

al., 2004). Moreover, adipose tissue is a potent producer of cytokines, especially TNFα and IL-6, and in the general population increases in this tissue associate with increased levels of circulating cytokines (Pi-Sunyer, 2006). Thus, increases in BF might also affect disease activity of RA patients. The potential associations between adiposity of RA patients with CVD risk and disease activity form the main questions of the present project and are discussed later in the literature review following an introduction to the basic concepts of obesity.

2.2 Obesity

2.2.1 Historical Concepts and Definition

Obesity (from Latin *obēsus* = stout, fat, or plump. *Ēsus* is the past participle of *edere* = to eat) (Onions *et al.*, 1996) is a condition that develops from a chronic quantitative imbalance between energy intake and energy expenditure leading to accumulation of excessive adipose tissue (i.e. fat) within the body (Bray and Bellanger, 2006). According to the guidelines of the World Health Organisation (WHO, 2000) individuals with a body mass index (BMI) (i.e. weight divided by height squared; discussed in further detail in the following section) of more than 30kg/m^2 are classified as obese. They are a distinct category from those with a BMI of 25-30kg/m^2 who are characterised as overweight.

Obesity has been recognised as a medical condition at least since the time of Hippocrates. He wrote “Corpulence is not only a disease itself, but the harbinger of others”, recognising that obesity is a medical disorder also leading to several comorbidities (Haslam and James, 2005). The first attested use of the word in English is in 1651, in *Matæotechnia Medicinæ Praxeos* by Noah Biggs (Onions *et al.*, 1996). In the ancient times, obesity was considered a flaw and in the Christian religion excessive consumption of food was viewed as a sign of sloth and lust (Woodhouse, 2008). In recent years, obese individuals have been the target of social discrimination; they are less likely to be hired for a job, they earn less and they are less likely to be promoted (Haslam and James, 2005). On the same line, the perceived ideal weight has changed during the past century. Indicative is the trend in “beauty contests” as the weight of the winners has decreased by 12% from 1922 to 1999 (Caballero, 2007).

Even though obesity was present since the early days of human civilisation, it is only in the last few decades that it has become a serious problem. For the most of human history, mankind was faced with starvation. During the industrial revolution it was realised that the efficiency and productivity of the workers depended on their body size and strength. Increased energy consumption resulted in height and weight increases throughout the 19th century in the developed world. However, as populations reached their genetic potential for height in the 20th century, weight increased disproportionately, resulting in obesity. By the year 2000, the human race reached a negative landmark, the number of adults with excess weight (i.e. BMI >25kg/m^2) is now higher than that of normal-weight (i.e. BMI between 18.5kg/m^2 and 25 kg/m^2) (Caballero, 2007).

2.2.2 Assessments

The assessment of obesity is a complicated procedure as direct measurement of the adipose tissue *in vivo* is virtually impossible. Thus several indirect methods for its assessment have been devised.

2.2.2.1 Body Mass Index

BMI is the most commonly used method for the assessment of obesity in the clinical setting and in epidemiological studies (WHO, 2000). It was developed in 1871 by Adolph Quetelet, a Belgian statistician and anthropometrist and for that it is also known as Quetelet index (Eknoyan, 2008). BMI is a simple mathematical calculation based on height and weight; depending on the units used this calculation is:

- *System International units:* BMI = weight (kg) / height^2 (m)
- *Imperial units:* BMI = weight (lb) x 703 / height^2 (in)

BMI is inherent in the definition of obesity as the world health organisation (WHO) has used this measure to categorise individuals according to their body weight. The categories and the corresponding BMI values are listed in Table 6.

Table 6: Body mass index categories for the general population

BMI (kg/m^2)	**Category**
<18.5	Underweight
18.5-25	Normal Weight
25- 30	Overweight
>30	Obese

These categories, in general, associate with low, medium, and high risk for health complications (WHO, 1998, Calle *et al.*, 1999, Haslam and James, 2005, Eknoyan, 2008). However, BMI is only a proxy of BF (Wellens *et al.*, 1996) and over recent years its validity has been questioned (Manson *et al.*, 1995, Willett *et al.*, 1995, Blew *et al.*, 2002, Nevill *et al.*, 2004, Romero-Corral *et al.*, 2006). Overweight as defined by BMI of >25, has poor specificity in detecting excess BF in males and in the elderly (Wellens *et al.*, 1996) as well as in patients with coronary heart disease (Romero-Corral *et al.*, 2006). In specific sub-populations, such as Indian-Asian (WHO, 2004), women (Manson *et al.*, 1995, Willett *et al.*, 1995, Blew *et al.*, 2002), and large size athletes (Nevill *et al.*, 2004), new BMI cut-off points have been suggested, that optimally reflect BF and may better predict CVD risk.

The weakness of BMI is that it does not distinguish between lean-body mass and fat mass. Consequently people of similar stature and weight, but

different muscle content, will have the same BMI but different levels of BF. This tends to be more evident in individuals with low BMI levels (Wellens *et al.*, 1996). Such limitations of BMI may explain the better cardiovascular outcomes observed in overweight and mildly obese patients with established CVD as compared to their normal-weight counterparts, who may have proportionately more BF (Romero-Corral *et al.*, 2006). Therefore, although it is well established that CVD risk increases with advancing BMI levels, (WHO, 1998) global cut-off points may be misleading for several populations.

2.2.2.2 Central Obesity

Central obesity, also termed "apple-shaped" or intra-abdominal obesity, refers to the accumulation of fat in the visceral area (fat deposited between the internal organs in the torso). Increased intra-abdominal fat results in an increased waist size and associates with negative health outcomes (Yusuf *et al.*, 2005). Even though not indicative of total body fat content, assessments of central obesity correlate strongly with cardiovascular disease (Hsieh and Yoshinaga, 1995, Rimm *et al.*, 1995). Waist circumference and waist-to-hip ratio are the two most widely used such assessments. Gender specific cut-off points for both are designed to identify individuals at increased risk for CVD (Table 7).

Table 7: Cut-off points for waist circumference and waist-to-hip ratio

Waist Circumference		Waist-to-hip ratio	
Male	Female	Male	Female
102 cm	88 cm	0.9	0.85

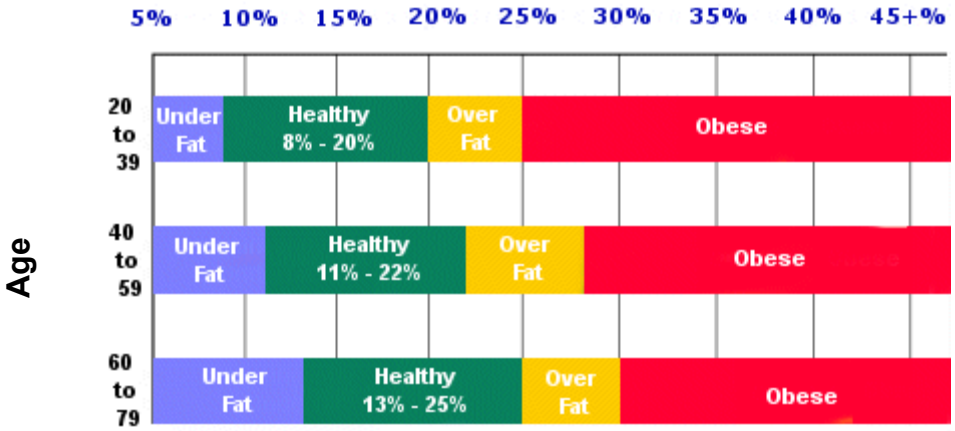
Several studies have proposed that, compared to BMI, central obesity better identifies individuals at increased risk for CVD (Janssen *et al.*, 2002, Janssen *et al.*, 2004) and thus the definition of obesity should be based on it. However, its predictive strength can be negatively affected by gender and overall body weight. Pear- shaped or obese individuals might have optimal waist circumference or waist-to-hip ratio but increased overall body fat (Li *et al.*, 2006). More research is necessary to identify the optimal definition of obesity as a predictor for CVD in the general population and specific sub-groups (Bray, 2004).

2.2.2.3 Body Fat Percentage

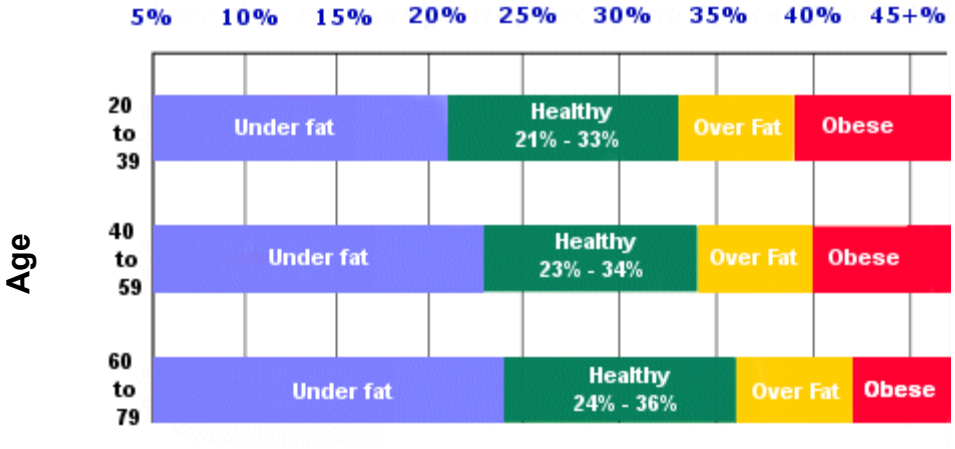
BF is an indicator of total body fat content and refers to both essential (i.e. the minimum amount of fat necessary for maintenance of life and reproductive function) and storage (i.e. the excess amount of fat stored in the various adipose depots of the body) fat. In men essential fat is 2-5% whereas in women 10-13% (Bray *et al.*, 1998). Storage fat can be accumulated either in subcutaneous (i.e. below the skin) or in visceral (i.e. around internal organs in the chest and abdomen) adipose tissue. Levels of optimal BF are age and gender specific (Figure 1) (Bray *et al.*, 1998).

Figure 1: Recommended body fat levels according to gender and age (Adopted from: WHO,2000)

Male



Female



Estimation of BF is a complicated procedure and several different methods have been proposed. Some methods have been based on the assessment of body density. Fat is known to have lower density (0.9kg/L) than other organs (1.07kg/L) and bones (1.9kg/L) (Visser *et al.*, 1997), thus estimation of average body density can reveal BF. Skinfold measurement and hydrostatic or underwater weighing are the two most widely used such methods. The former involves measurement of different skinfolds of the body (i.e. biceps, triceps, subscapular, iliac crest, supraspinale, abdominal, front thigh, median calf) with the use of a calliper (ACSM, 2005). The sum of skinfolds is then input in specific equations (for details refer to Jackson *et al.*, 1980, Jackson and Pollock, 2004) to estimate body density. Then, with the use of yet another equation (Siri, 1961) BF can be estimated. This technique however is not considered highly valid especially in non-athletic populations as it does not take into account visceral fat (ACSM, 2005). Hydrostatic weighing, on the other hand, is considered to be a highly valid method for assessing BF and until recently it was the “golden standard” against which newer methods were validated (Brodie, 1988). Hydrostatic weighing involves assessment of body weight in and out of the water. Due to its low density, fat is lighter than water and thus it floats. The two weight measurements together with lung residual volume and water density are input in a formula that calculates again body density (Warner *et al.*, 1986). BF is then calculated again with the Siri equation (Siri, 1961). Air displacement plethismography also calculates body density and from that BF (Fields *et al.*, 2002). With the advancement of technology several other more direct methods for the assessment of BF have been developed.

Near Infrared Interactance measures the thickness of subcutaneous fat over the biceps and, based on equations, BF can be estimated. This method assumes that body fat distribution is universal in the body thus might underestimate BF of individuals with central obesity (Wagner and Heyward, 1999).

Total body potassium assesses the subject's natural radiation, as potassium-40 is measured through the use of scintillation counters. This procedure accurately determines the body's total cell mass which in turn can be used to estimate fat-free or lean body mass. This method is highly valid and reliable; however, the cost of the equipment is a major deterrent for its use.

Total body nitrogen is a similar technique that measures the amount of nitrogen in the body and is a direct indicator of total body protein (Brodie, 1988). Nitrogen and protein are closely linked with each other because of a stable chemical combination (protein is 16% nitrogen) and because over 98% of the total body nitrogen is in the form of protein (Heymsfield *et al.*, 1993). This technique is again highly valid but cost of the equipment is also high (Haas *et al.*, 2007).

Computed tomography is a common medical procedure used to diagnose several disorders. It produces high quality images of body tissues and organs which can be used to differentiate and measure the amounts of fat and lean body tissue, and further distinguish between intra-abdominal and extra-abdominal fat. This method is one of the most valid as it literally "photographs" adipose tissue (Rogalla *et al.*, 1998). However, the exposure to high amounts of radiation (1 tomography = 500 chest x-rays) and the cost of the test limit its use in clinical or research settings (Wagner and Heyward, 1999).

Magnetic resonance imaging is a method with many similarities to computed tomography. It also "photographs" adipose tissue and allows for estimation of total and regional fat mass and at the same time it is safer since no radiation is involved (Tothill and Stewart, 2002). However, the extreme cost of the equipment and its continuous use for other assessments limits its availability for research (Wagner and Heyward, 1999).

Dual energy X-ray absorptiometry (DEXA) is a method initially aimed at assessing bone mineral density. However, several studies have found it to be a valid and reliable method for assessing BF (Wagner and Heyward, 1999, Bolanowski and Nilsson, 2001, Glickman *et al.*, 2004). DEXA differentiates body weight into the components of lean soft tissue, fat soft tissue and bone, based on the differential attenuation by tissues of two levels of x-rays. The estimation of total and regional BF is thus based on a three-compartment model (bones, lean and fat mass) making DEXA the current reference method for BF assessment (Glickman *et al.*, 2004). Its very low radiation and its relatively low cost (compared to CT and MRI) make it also very popular with researchers. However, the long assessment time (>15 minutes) does not allow for clinical use.

Bioelectrical Impedance Analysis (BIA) is a relatively new method used for the estimation of BF in different populations. BIA devices emit a low-intensity electrical current that passes through the body. Electrodes placed at different sites on the body assess the resistance to the current. Due to differing water and electrolyte content BF produces a higher resistance compared to fat free mass (FFM); thus a high resistance indicates increased BF levels (Bolanowski and Nilsson, 2001). Devices with several electrodes are able to send the electrical current through different parts of the body and assess segmental body

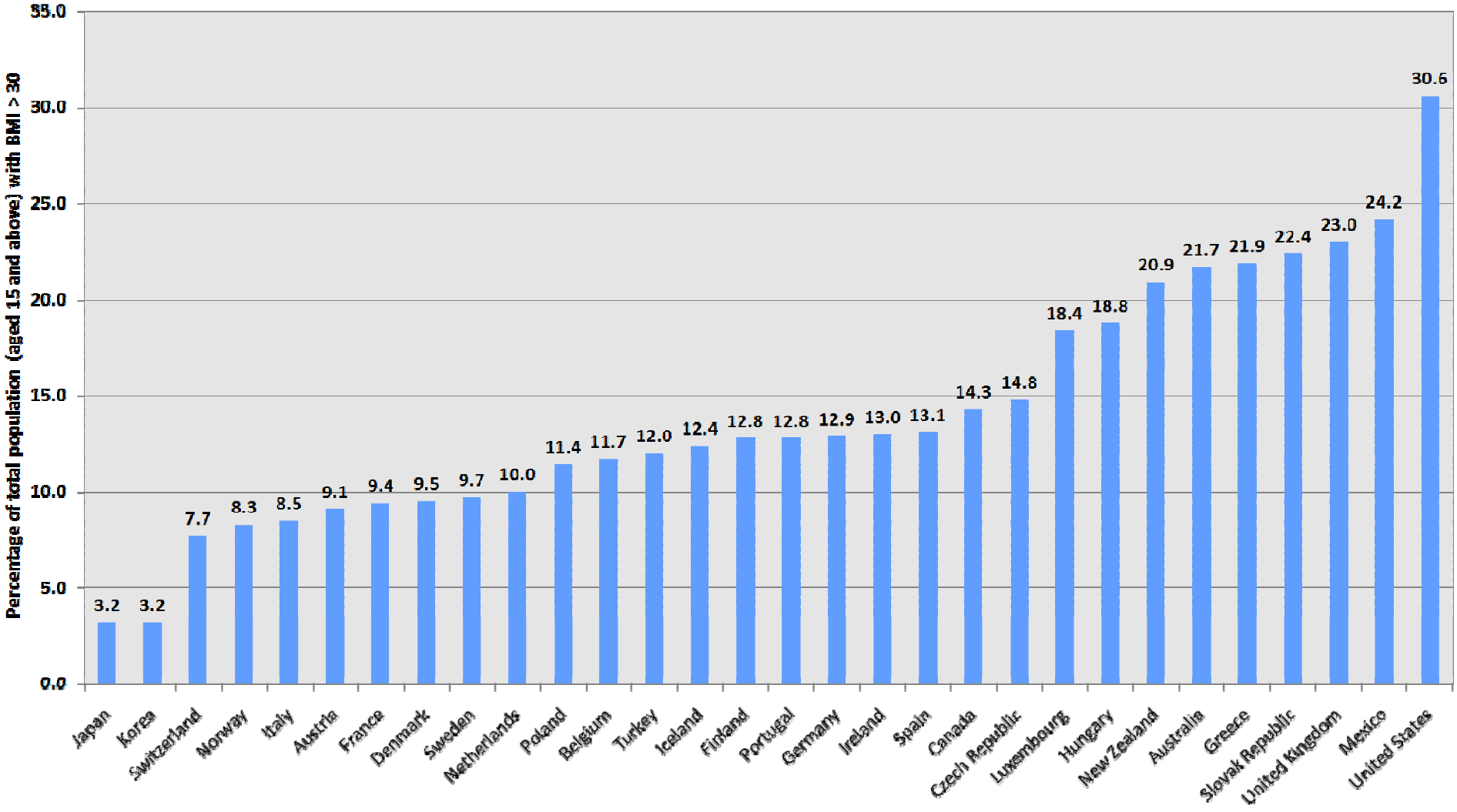
composition (Demura *et al.*, 2005). This method has become popular and widely recommended, as it is reliable, objective, practical, relatively inexpensive, and does not require highly trained personnel (Demura *et al.*, 1999, Demura *et al.*, 2004). The validity of this method has been confirmed in various studies (Gray *et al.*, 1989, Oppliger *et al.*, 1992, Tanaka *et al.*, 1999, Bolanowski and Nilsson, 2001, Demura *et al.*, 2004). Especially devices with multiple electrodes using single frequency electrical current generate highly reproducible measurements of total BF and segmental fat distribution (Demura *et al.*, 2005, Ishiguro *et al.*, 2006). Their correlation with the "gold standards" of dual-energy x-ray absorptiometry and hydrostatic weighing is 0.90 and 0.80 respectively, with a standard error of around 3.0, producing a co-efficient of variation of <10% (Demura *et al.*, 2004). This suggests that bioelectrical impedance measurements are valid and suitable for body composition studies (Bolanowski and Nilsson, 2001, Demura *et al.*, 2004, Demura *et al.*, 2005). Patients are usually happy to undergo such a measurement due to its simplicity and similarity to normal weighing.

2.2.3 Epidemiology

Until recently obesity was considered a condition affecting only residents of developed countries especially in the United States and Europe. In recent years however, obesity has become a world wide problem (WHO, 2000) increasing more rapidly in developing countries (Popkin and Gordon-Larsen, 2004). Its global nature was first realised by the WHO in 1997 (WHO, 1998) (Figure 2), and by 2000 more than 1.1 billion people worldwide were overweight while more than 400 million were obese (WHO, 2000). Almost 30.000 deaths per year are currently attributed to obesity and with the significant increases in its prevalence it is projected that obesity will overtake smoking as the major avoidable cause of premature death by 2030 (Haslam *et al.*, 2006). Obesity has also significant economic costs as it results in almost 18 million days of absence from work due to illness each year; it also associates with increased hospitalisation and medical costs (Haslam *et al.*, 2006).

Specifically in the UK, 23% of men and 25% of women are obese (Rennie and Jebb, 2005). When overweight individuals are taken into account, almost 70% of males and more than 50% of females show BMI above 25kg/m^2 (National Audit Office, 2001). Even though obesity increases with age, almost 20% of children under the age of 15 and almost 30% of young adults (15-20 years of age) are overweight or obese (Department of Health, 2002).

Figure 2: Prevalence of obesity in several different countries



Adopted form: Organisation for Economic Co-operation and Development (http://www.oecd.org/home/)
Percentage of total population (aged 15 and above) classified as obese (BMI> 30kg/m^2). Depending on country, data were collected and published between 1996 and 2003.

2.2.4 Aetiology

Obesity is the result of a combination of genetic, behavioural, environmental, physiological, social, and cultural factors that affect energy balance and promote excessive fat deposition.

2.2.4.1 Genetic Susceptibility

Several studies have reported a familial trend of obesity. Children of lean parents are less likely to be overweight or obese; whereas those of obese parents are more likely to be obese as children or become obese in their adult life (Treuth *et al.*, 2003). However, it is not entirely clear whether this is a result of genetics or merely of the lifestyle habits of the family. Children of obese individuals tend to consume more energy and exercise less compared to those of lean parents (Wardle *et al.*, 2001).

Nevertheless, in studies of adoptees, the weight class (i.e. underweight, normal weight, overweight or obese) of the adopted children in adulthood was strongly related to the BMI of their biological parents, but it was unrelated to the BMI of their adoptive parents indicating a stronger influence of genetics rather than the environment (Stunkard *et al.*, 1986). Similar conclusions were reached in a study of homozygotic twins. Following a 100-day overfeeding, twins gained body weight ranging from 4.3-13.3kg. The variance in response was significantly lower within pairs of twins rather than among pairs. The similarity in response within pairs was evident for body weight, percentage of fat, fat mass, and estimated subcutaneous fat (Bouchard *et al.*, 1990). In other words, some twin pairs gained much more weight than other pairs. The concordance in response between

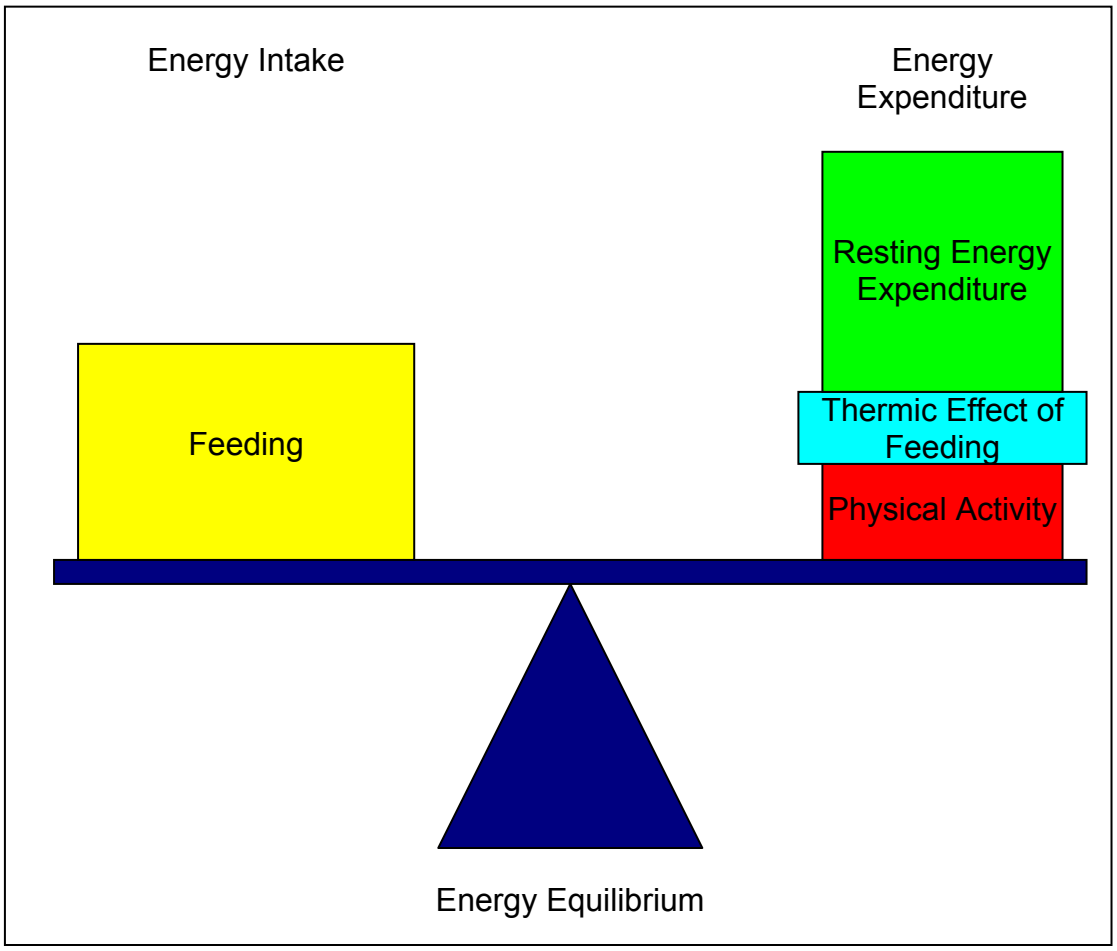
identical twins demonstrates the impact of genetics on weight gain (Pi-Sunyer, 2002).

Up to date 22 genes have been shown to affect body weight (i.e. more than five positive studies for each gene), 127 candidate genes have been proposed as possible confounders (i.e. less than five positive studies) and several hundred mutations of these genes have been identified (Rankinen *et al.*, 2006). However, one gene has shown consistent associations with both the development and the severity of obesity: the FTO gene (Dina *et al.*, 2007, Frayling *et al.*, 2007, Gerken *et al.*, 2007, Scuteri *et al.*, 2007, Fredriksson *et al.*, 2008). This gene associates with an almost two-fold increased risk for obesity while carriers of a specific mutation weigh an average 3kg more than those carrying different mutations (Frayling *et al.*, 2007). Worryingly, the prevalence of the weight-gain prone mutation is 45% among Europeans (International HapMap Consortium, 2003). Despite its wide prevalence and its strong association with obesity, the FTO gene or any other gene (or their combination) indeed, can not explain the steep increase in obesity rates of the last decades. Population-wide genetic alterations do not occur in the relatively short period of time during which obesity reached epidemic proportions (Racette *et al.*, 2003).

2.2.4.2 Energy Equilibrium

According to the first law of thermodynamics (i.e. energy can neither be created nor destroyed, it can only change forms), the only way to accumulate excess body weight is through a positive energy balance. Even the slightest changes in energy equilibrium when sustained for a sufficient period of time can result in marked changes in body weight (Figure 3).

Figure 3: Energy equilibrium (McArdle et al., 2001)

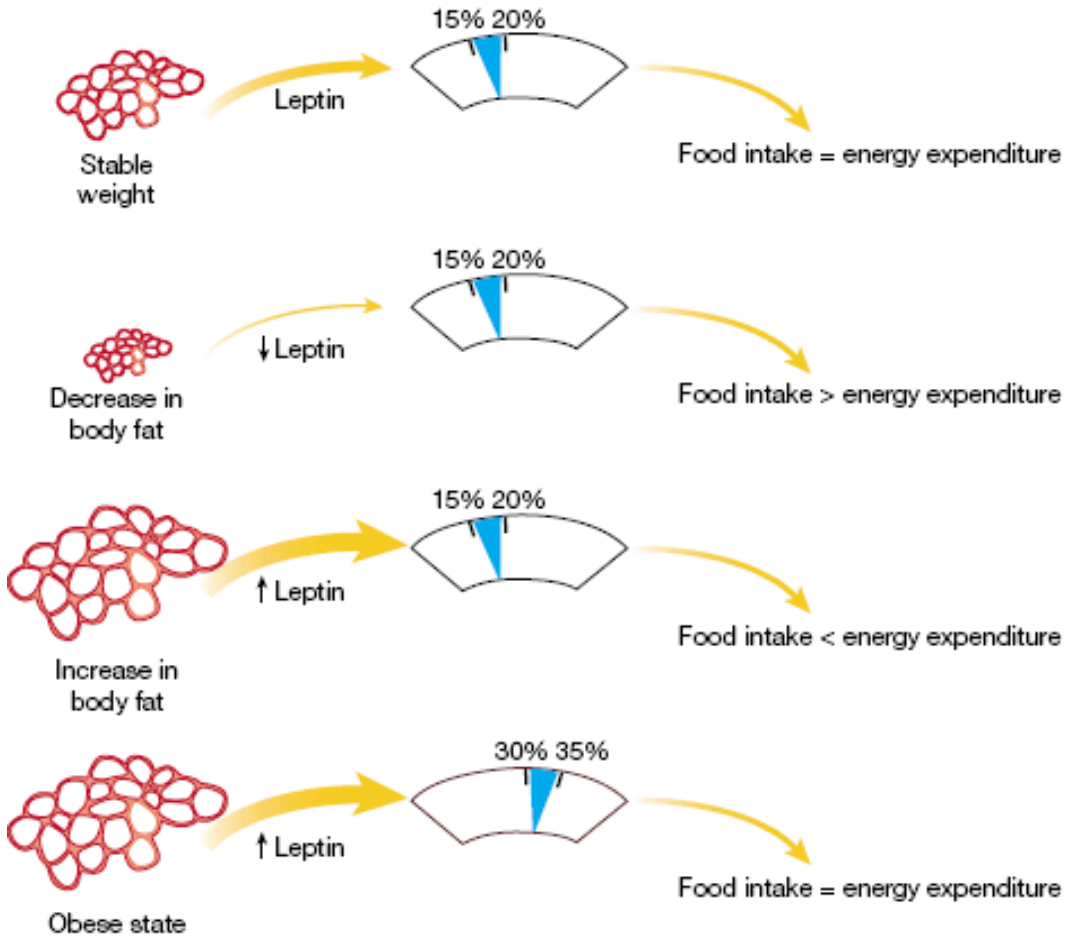


When energy intake equals energy expenditure, body weight remains unchanged. When intake exceeds expenditure body weight increases whereas when expenditure exceeds intake, body weight decreases.

A surplus of only 10 calories per day (i.e. almost two grams of carbohydrates or 1 gram of fat) would result in an annual increase of body weight by almost 0.5kg. If this imbalance was sustained throughout adulthood, it would result in significantly increased body weight by the age of 40-50 (Racette *et al.*, 2003). However, the control of energy intake and expenditure is a complicated procedure. In order to maintain body weight over the years, the two need to match within 0.17% per decade (Weigle, 1994). For that level of precision to be achieved, tight regulation of energy intake and expenditure is necessary.

Several different mechanisms by which the body maintains energy homeostasis have been proposed, however the most potent and widely accepted is through leptin (Friedman, 2000). Leptin (from Greek: *λεπτός* meaning thin) is a hormone-like protein that regulates appetite and energy expenditure (Caro *et al.*, 1996) by reporting nutritional information to the hypothalamus (Friedman, 2000) (Figure 4). It is secreted by and in proportion to the adipose tissue mass (Considine *et al.*, 1996). An increase in adipocyte size increases levels of circulating leptin leading to a reduction in appetite and increase in energy expenditure. On the contrary, a reduction in adipocyte size decreases leptin concentration resulting in increased appetite and reduced energy expenditure (Considine *et al.*, 1996). Its secretion is also affected by short-term food intake (Racette *et al.*, 2003). Increased food intake increases levels of circulating leptin while decreased food intake decreases them (Kolaczynski *et al.*, 1996, Racette *et al.*, 1997). This mechanism allows for the required tight regulation of energy balance; however, obesity is considered a state of leptin resistance. Most obese individuals exhibit high levels of leptin while their energy intake remains increased and their energy expenditure reduced (Friedman, 2000). Whether leptin resistance is genetically controlled or it depends on environmental factors is not known, however most researchers suggest a significant influence of continuous increased energy intake that renders the hypothalamus “immune” to leptin (Racette *et al.*, 2003).

Figure 4: Interactions between adipose tissue and leptin in the control of energy intake and expenditure



Adopted form: Friedman, 2000; p.632. At stable weight (shown as15–20% body fat in this figure, which is the typical fat content of a non-obese male subject) the amount of circulating leptin elicits a state in which food intake equals energy expenditure. Increasing leptin levels result in negative energy balance (energy expenditure < food intake), whereas decreasing levels lead to positive energy balance (food intake > energy expenditure). These effects maintain constancy of fat cell mass within a relatively narrow range. Evidence further suggests that the intrinsic sensitivity to leptin is reduced among the obese and that the set point for body fat content is thus increased (designated as 30–35% in the bottom panel). Most obese individuals have high leptin levels and thus enter a state of negative energy balance when weight is reduced and leptin levels fall (Friedman, 2000).

2.2.4.3 Energy Intake

In modern society a large number of eating establishments provide a large variety of inexpensive, tasty but most importantly time-saving meals. Food manufacturers strive to enhance the appearance, flavour, and portion size of their products and, through advertisement, promote over-consumption (Racette *et al.*, 2003). The ease of access and omnipresence of such food, which often is of high calorific but low nutrient content, leads to the belief that its consumption is safe and healthy, diverting people away from cooking and eating at their own place (McCrory *et al.*, 1999). This has led to a small but significant increase in energy intake over the past 20 years. Dietary surveys and food disappearance data indicate an increase in caloric intake in Westernised countries of about 200kcal/week (Nielsen *et al.*, 2002). A large proportion of these excess calories comes from the consumption of sweetened beverages and dietary fats which account for almost 25% total daily intake among young adults (Rajeshwari *et al.*, 2005, Striegel-Moore *et al.*, 2006). At the same time the consumption of "healthy" calories, derived from fruits and vegetables, remains below recommended levels (Caballero, 2007).

However, the extent to which the above listed changes in eating habits are responsible for the obesity epidemic is still under intense debate. The increase in daily caloric intake is partly counterbalanced by the increases in height (leading to increased energy requirements by the body) observed in the population globally over the past decades (Caballero, 2007). Moreover, fat intake over the last decades has decreased and its associations with body fat are not consistent (Willett and Leibel, 2002). Thus several authors suggest that what has changed in terms of energy intake is not the amount of food or type of nutrients but the ease of access to it (Racette *et al.*, 2003, Rosmond, 2004, Caballero, 2007).

2.2.4.4 Energy Expenditure

Total daily energy expenditure (TDEE) is composed of three major aspects: resting energy expenditure (REE), thermic effect of feeding (TEF) and thermic effect of exercise (TEE). REE is the amount of energy required by the body to maintain physiological function and accounts for almost 70% of TDEE in sedentary individuals (Poehlman, 2002). It is largely dependent on the amount FFM (i.e. muscle and organ mass) with active individuals exhibiting both more and better quality FFM (Poehlman, 1989, Poehlman *et al.*, 2002). TEF is the amount of energy required by the body to process food and store nutrients and depending of meals size, it accounts for 10-15% of TDEE (Swaminathan *et al.*, 1985). TEF increases linearly with increasing calorific content, but it is relatively independent of the type of food (D'Alessio *et al.*, 1988). However, obese individuals are suggested to have limited TEF in response to fat ingestion (Swaminathan *et al.*, 1985). TEE is the most changeable component of TDEE. It accounts for 20-30% of TDEE in sedentary individuals but may reach up to >50% in athletes or exercising individuals (Poehlman, 1989). In addition to the direct increases in TEE, exercise is also known to increase REE over a period of 24-72hours resulting in even greater TDEE (Stavropoulos-Kalinglou, 2002). Exercise is also an effective means of increasing FFM, thus resulting in long-term REE increases as well (Poehlman, 1989). As such exercise is the major contributor to changes in TDEE and even small alterations in it might affect positively or negatively energy balance (Poehlman *et al.*, 2002).

In modern society however, industrialisation and the numerous technological advances have enabled humans to evolve from hunter-gatherers to highly sedentary individuals; they rely entirely on several different machines that

perform, once energy-consuming, daily activities. Similarly, changes in the educational systems and family structure have led to reductions in physical education in schools and to increases in the number of children who stay indoors after school (Racette *et al.*, 2003). This promotes sedentary habits such as television watching or use of video-games, instead of physical activity (Whitaker *et al.*, 1997), which are also accompanied by consumption of snack-like diet (Kubo *et al.*, 2008). These changes have coincided with the increases in the prevalence of obesity and appear to be the most potent explanation for it. However, obesity is most likely a result of a combination between genetic predisposition and lifestyle with a relative contribution of 30% and 70% respectively (Pi-Sunyer, 2002).

2.2.5 Disorders Associated with Obesity

Obesity predisposes to a large number of different health conditions and also associates with increased mortality (Calle *et al.*, 1999). Life expectancy of obese individuals is decreased by 6-7 years and due to the large number of such individuals in the modern societies, some researchers have suggested that life expectancy in several countries could potentially be reduced for the first time in more that two centuries (Olshansky *et al.*, 2005). Known complications of obesity are listed in Table 8, however only its associations with CVD and inflammation are further discussed.

Table 8: Disorders associated with obesity (Bray *et al.*, 1998)

System	Condition
Cardiovascular	angina (RR= 1.8 both genders) myocardial infarction (RR= 1.5 males, 3.2 females) congestive heart failure (12% attributable to obesity) high blood pressure (RR= 2.6 males, 4.2 females) high cholesterol deep vein thrombosis and pulmonary embolism
Endocrine and reproductive	diabetes mellitus (RR= 5.2 males, 12.7 females) polycystic ovarian syndrome menstrual disorders infertility complications from pregnancy birth defects
Musculoskeletal	hyperuricemia / gout: (RR= 2 males, 3 females) immobility osteoarthritis (RR= 1.9 males, 1.4 females) low back pain
Neurological	stroke (RR= 1.3 both genders) headache carpal tunnel syndrome dementia idiopathic intracranial hypertension
Cancers	breast esophageal colorectal (RR= 3 males, 2.7 females) liver gallbladder (RR= 1.8 both genders) pancreatic stomach, prostate endometrial, cervical ovarian (RR= 1.7 females) kidney non-Hodgkin's lymphoma multiple myeloma
Gastrointestinal	gastro-esophageal reflux disease fatty liver disease cholelithiasis hernia
Respiratory	obstructive sleep apnea obesity hypoventilation syndrome asthma complications from general anaesthesia
Psychological	Depression in women low self esteem body dysmorphic disorder social stigmatization suicide (RR=2 both genders)

Skin	stretch marks acanthosis nigricans lymphedema cellulitis carbuncles intertrigo
Genitourinary	erectile dysfunction urinary incontinence chronic renal failure hypogonadism stillbirth

RR= relative risk

2.2.5.1 Cardiovascular Disease

CVD is probably the most readily recognisable effect of obesity. Obese individuals are at significantly higher risk for developing CVD compared to their lean counterparts. The exact links between obesity and CVD are not clear. However, the associations between obesity and classical CVD risk factors (i.e. hypertension, dyslipidaemia and insulin resistance) are well established. Excess adipose tissue releases nonesterified fatty acids in the circulation, which overload the liver and muscles with lipids. This increases lipolysis, while reducing glucose uptake and utilization by the cells. The resulting increase in circulating glucose stimulates insulin production, and eventually leads to insulin resistance (Bray *et al.*, 1998). Endothelial function is also impaired causing arterial stiffness and hypertension (Zizek *et al.*, 2001). Obesity may also increase CVD risk by reducing adiponectin. Adiponectin is a hormone secreted by adipocytes responsible for glucose regulation and fatty acid catabolism. Decreased levels of this hormone have been associated with insulin resistance and hyperlipidaemia (Ukkola and Santaniemi, 2002). Other mechanisms by which adiposity might increase CVD risk is the activation of the rennin-angiotensin-aldosterone system, which in turns increases sympathetic activity and renal sodium reabsorption leading to hypertension (Bray *et al.*, 1998).

2.2.5.2 Inflammation

In recent years adipose tissue has been recognised as a potent endocrine and paracrine organ capable of synthesizing and releasing into the bloodstream an important variety of peptides and non-peptide compounds that may play a role in cardiovascular homeostasis and inflammation (Mohamed-Ali *et al.*, 1998). Among others, adipose tissue is a significant source of TNFα, IL-6, leptin and adiponectin (Poirier *et al.*, 2006). Increases in BMI result in higher levels of circulating TNFα, IL-6, and leptin and lower levels of adiponectin; reductions in BMI have the opposite results. *In vivo*, almost 30% of the total circulating concentrations of IL-6 originate from adipose tissue (Poirier *et al.*, 2006). This is of importance because IL-6 modulates CRP production in the liver (Yudkin *et al.*, 1999), and CRP (which is elevated in obesity) may be a marker of a chronic inflammatory state that can trigger acute coronary syndrome (Ridker, 2007).

The mechanism by which increased adiposity results in inflammation is not clear; however adipose hypoxia seems to be the most likely explanation. In order to facilitate fat storage, adipocytes have to expand in size, as differentiation of pre-adipocytes to adipocytes in this tissue is limited. Enlarged adipocytes show consistent low-grade inflammation (Matsuzawa, 2005). Recent studies have attributed this to localised tissue hypoxia (Trayhurn and Wood, 2004, Hosogai *et al.*, 2007, Ye *et al.*, 2007). Adipose tissue has limited vascularisation and a small number of mitochondria (Ailhaud and Hauner, 1998) resulting in its relatively poor oxygenisation; enlargement of adipose cells further limits oxygen delivery to them (Trayhurn and Wood, 2004). This results in a dysregulation of the expression and secretion of pro- and anti- inflammatory cytokines (i.e. increased IL-6 and TNFα and decreased adiponectin) (Hosogai *et al.*, 2007, Wang *et al.*, 2007, Ye *et al.*,

2007, Trayhurn *et al.*, 2008). Increased levels of pro-inflammatory molecules have been implicated in the process of atherosclerosis (Ross, 1999), specifically by inducing endothelial dysfunction (Alexander, 1995, Bhagat and Vallance, 1997)- the first step of atherosclerosis.

2.2.5.3 Subgroups of Obesity

However, not all obese individuals exhibit increased CVD risk or inflammation and not all normal-weight individuals are metabolically healthy or free from CVD (Karelis *et al.*, 2004). Two distinct subtypes of obesity have been identified (Karelis *et al.*, 2004). The first subtype, termed metabolically-obese but normal-weight (MONW), consists of individuals with normal weight who exhibit increased levels of insulin resistance and accumulation of other modifiable CVD risk factors (i.e. hypertension, dyslipidaemia) (Ruderman *et al.*, 1981, Ruderman *et al.*, 1982). The second subgroup, termed metabolically-healthy but obese (MHO), exhibits increased levels of body mass index (BMI) and body fat (BF) but no other metabolic complications (Sims, 2001). Significantly, both subtypes associate with different inflammatory profiles. MONW exhibit increased levels of inflammation compared to other normal-weight individuals (Hyun *et al.*, 2008), while MHO exhibit reduced levels of inflammation compared to other obese individuals (Shin *et al.*, 2006, Karelis and Rabasa-Lhoret, 2008).

2.2.6 Control of Obesity

The aims of obesity treatment are to achieve and then maintain clinically significant weight loss, with the ultimate target of reducing the risk for, or severity of, obesity related diseases (Racette *et al.*, 2003). Even a 5% reduction in initial body weight has been shown to significantly improve overall health and the risk for further complications (Volek *et al.*, 2002, Wilson and Grundy, 2003). However, long-term success requires maintenance of a 10% reduction in body weight over a period of at least one year. Even though initial weight loss is difficult, its maintenance is an even greater challenge both for health professionals and for patients themselves; only 1/5 of the patients who achieve initial weight loss manage to retain it for the first year and even less for longer periods of time (Wing and Hill, 2001). Thus any weight loss regimen should be designed according to the individual needs of each patient and utilise all necessary modes of weight reduction (Racette *et al.*, 2003).

2.2.6.1 Diet

Dieting is probably the most widely utilised mode of weight reduction and forms the basis of most weight loss regimens. Diets attempt to provide a balanced nutrition focusing on increased nutrient intake (e.g. fibres, vitamins) and reduced caloric value of meals (Ayyad and Andersen, 2000). Their success depends highly on the total amount of calories they prescribe. Diet can be divided in two large categories: very-low-calorie diets (VLCDs, <800kcal/day) and low-calorie-diets (LCDs, 800-1500kcal/day). VLCDs are very effective in rapidly reducing weight; they result in a 2000kcal/day energy deficit which leads to a weight-loss of almost 2kg per week (Orzano and Scott, 2004). Despite their short-term benefits, VLCDs

are only used in extreme cases of obesity or when rapid weight-loss is required (i.e. before an operation). They associate with a number of serious side-effects, such as gout, cholelithiasis, and hair loss, and long-term maintenance of weight-loss is generally poor (Ayyad and Andersen, 2000). LCDs, on the other hand, are more commonly used. The modest restriction of energy intake results in 0.5-1kg weight loss per week and can be retained for longer periods (Orzano and Scott, 2004). Such diets can be easily incorporated in the lifestyle of patients increasing adherence. In the long-term, a greater and more easily sustainable weight-loss compared to VLCDs is achieved (Racette *et al.*, 2003).

Both dieting modes have the same drawback. Any energy restriction regimen results in a decrease of FFM (Ravussin *et al.*, 1985); the decrease is proportional to the energy deficit (Mingrone *et al.*, 2002). As stated previously, FFM is major contributor to REE; reductions in the former will decrease the latter. Consequently, since REE is the larger component of TDEE, this will result in a major decrease in TDEE. For the diet to maintain its effectiveness, energy intake needs to be constantly reduced. This might lead to cases of severe undernutrition and starvation (Racette *et al.*, 2003) even among the obese (Mingrone *et al.*, 2002). Thus, in addition to individual design of dieting regimens, an intervention that maintains FFM and increases TDEE should be present during weight-loss.

2.2.6.2 Exercise

Exercise is the only such intervention. It is known to elicit both direct and indirect effects on energy expenditure. It directly increases TEE and indirectly REE (Poehlman, 1989, Poehlman, 2002, Poehlman *et al.*, 2002, Stavropoulos-Kalinglou, 2002). Several studies have investigated the weight reducing potential of exercise; however most have failed to notice significant changes in body weight

(Miller *et al.*, 1997, Jakicic *et al.*, 1999). The main reason for that is the short duration of the interventions (Donnelly *et al.*, 2003) and also their complete reliance on BMI to assess obesity (Miller *et al.*, 1997). Exercise in sedentary individuals can increase TDEE by an average of 300-500kcal/day resulting in almost 1kg weight-loss per month (The Look AHEAD Research Group, 2003). Thus, substantial changes in body weight require longer interventions. Nevertheless, what most studies have not assessed, are alterations in body composition. The reason that total weight does not change significantly is that while reducing fat, exercise also increases FFM. Because FFM is heavier that fat (McArdle *et al.*, 2001), BMI seems unchanged or even slightly increased, especially in individuals who partake in resistance exercise (Nevill *et al.*, 2004). Despite the lack of change in BMI, and most likely due to its effect of body composition, exercise has been shown to reduce risk for obesity-related diseases as well as inflammation in overweight and obese individuals, irrespective of weight change (Wood *et al.*, 1988, Ross *et al.*, 2000, Donnelly *et al.*, 2003, Jeffery *et al.*, 2003).

In order for exercise to elicit its beneficial effects on body weight and composition, a minimum intensity and duration has to be achieved. For effective weight reduction, physical activity should produce a weekly energy expenditure of at least 2500kcal. The currently accepted guidelines for weight control, advocate at least three exercise sessions per week, of 45-60min duration at an intensity of >60% of an individual's aerobic capacity (ACSM, 2005). However, fitting more than three hours of exercise in the very busy week of a modern person is usually hard and this can be the decisive factor for someone not to exercise (Lakka and Bouchard, 2005). For that reason, prescriptions involving different intensities and

durations of exercise have been utilised. Recent findings indicate that very short repetitive bouts of intensive exercise or indeed increases in habitual physical activity (i.e. the physical activity associated with everyday life and not purposeful exercise) are equally effective in producing the required energy expenditure for weight loss (Chambliss, 2005). While high intensity sessions are better suited for younger individuals and those without any significant medical conditions, increases in habitual physical activity are better tolerated by older people and those with medical conditions (Lakka and Bouchard, 2005). To further support the significance of habitual physical activity, habitually active people are less likely to develop obesity compared to their habitually inactive counterparts irrespective of participation in other forms of exercise (DiPietro, 1999). Thus, the newest interventions for the prevention and treatment of obesity, and related conditions, focus on increasing habitual physical activity and maintaining it at a high level throughout a person's life.

2.2.6.3 Medical Approaches

Medication for weight-loss is also available. It is usually reserved for individuals who have already attempted to lose weight through diet and exercise and have failed or when co-existing medical conditions require rapid weight-loss (Racette *et al.*, 2003). They are usually used in combination with lifestyle modification and seem to have an additive effect, in terms of both weight loss and weight maintenance thereafter (Franz *et al.*, 2007). To date, three anti-obesity agents are available: Sibutramine, Orlistat and Rimonabant. Sibutramine is a centrally acting serotonin-norepinephrine reuptake inhibitor, which acts by inhibiting the reuptake of norepinephrine, serotonin and dopamine, thereby inducing satiety (Aronne, 2007). Orlistat is a pancreatic lipase inhibitor which reduces fat absorption from

the intestine through inhibition of the hydrolysis of dietary fat into absorbable free fatty acids and monoacylglycerols (Aronne, 2007). Rimonabant is a cannabinoid type 1 receptor antagonist, which has been shown to reduce food intake via inhibition of the endocannabinoid system, which is known to play a role in the central and peripheral regulation of body weight and energy balance (Van Gaal *et al.*, 2005)

Surgery is the final resource for weight-loss. It is reserved solely for individuals with very high BMI (>40kg/m^2) or those with a BMI of >35kg/m^2 in the presence of other obesity-related comorbidities such as type-2 diabetes, sleep apnea, or heart disease (Brolin, 1996). There are several approaches to bariatric surgery, but all procedures are either malabsorptive, or restrictive. Malabsorptive procedures, most often referred to as “gastric bypass”, change the way the digestive system works. In these procedures the upper portion of the stomach is stapled to create a small (10–30 mL) reservoir that attaches directly to the jejunum via a Roux-en-Y limb (Deitel and Shikora, 2002). This reduces the capacity of the stomach resulting in limited food intake. Also bypassing the stomach and upper portions of the small intestine inhibits the absorption of some nutrients and calories (Racette *et al.*, 2003). Weight-loss is rapid and patients can lose up to 70% of their excess body weight within six months (DeMaria *et al.*, 2002, Papasavas *et al.*, 2002). More importantly though, results are maintained for several years (Mitchell *et al.*, 2001, Mathus-Vliegen, 2006, Pajecki *et al.*, 2007). Restrictive procedures, usually referred to as “gastric stapling”, involve installing a constriction band around the upper portion of the stomach, effectively reducing its capacity. Its effectiveness depends on the patient; drastic dietary modifications are necessary to avoid complications, rupture of the band or even

death. This procedure is also less effective compared to gastric bypass in weight-reduction thus it is not as common (Racette *et al.*, 2003). In general both procedures have severe side-effects. Steatorrhea, vitamin and mineral deficiencies, and osteoporosis are common (Sugerman, 2000). Also wound infections, incisional hernia, and anastomotic leak with peritonitis may occur and could require further surgery for their treatment (Racette *et al.*, 2003). Before a patient decides to undergo such surgery the risk and benefits should be clearly established; following surgery lifelong supplementation and medical follow-up is necessary (Racette *et al.*, 2003).

2.3 Obesity in Rheumatoid Arthritis

Most research in the field of RA has focused on disease prevention and treatment, mainly due to the strong associations of RA with joint problems and the resulting movement limitations (Kitas and Erb, 2003). The cardiovascular aspect of the disease has also attracted some attention with mainly observational studies assessing the associations of RA with CVD risk and outcome (Cobb *et al.*, 1953, Goodson, 2002, van Doornum *et al.*, 2002, Douglas *et al.*, 2006). Even though the exact cause for the increased incidence and severity of CVD in RA is not known, it is most likely a combination of genetic predisposition (Gonzalez-Gay *et al.*, 2007), modifiable CVD risk factors and the inflammatory burden of the disease (Gonzalez *et al.*, 2008).

Even though obesity is a significant contributor to CVD in the general population (Bray and Bellanger, 2006), it has received limited attention in RA. BMI is routinely assessed in most rheumatology clinics and consistently reported in RA research. However, it is mainly used as a demographic characteristic of the population and it is usually omitted form further analyses or interpretations. In the studies that include BMI in their analyses, it is considered as a confounding factor against which data should be corrected and not a primary or even a secondary outcome that could directly influence aspects of the disease or other conditions. Very few studies have addressed obesity as such and these studies are reviewed below.

2.3.1 Definition and Prevalence

Most of the studies assessing body weight in RA use the definition of the WHO (WHO, 2000) for overweight and obesity. Patients with a BMI between 25-30kg/m^2 are classified as overweight and those with a BMI>30kg/m^2 are classified as obese. Even though this definition is valid for the general population and can identify individuals at increased risk, it has been proven inaccurate for certain populations with altered body composition. For example, Asian-Indians exhibit increased levels of fat for a given BMI (WHO, 2004), while athletes exhibit increased levels of FFM (Nevill *et al.*, 2004). For the former group new BMI cut-off points that better identify individuals at increased risk for health complications have been established (WHO, 2004), while for the latter group BMI is considered an inaccurate measure of CVD risk (Nevill *et al.*, 2004). As discussed earlier (Section 2.1.7.2) RA associates with metabolic alterations that lead to reduction in FFM without any obvious change in total body weight. Thus, like Asian-Indians, RA patients should exhibit reduced FFM for a given BMI and the general BMI cut-off points might not be able to identify individuals with increased levels of BF. However, in all the studies reviewed herein, the general BMI cut-off points have been used to assess overweight and obesity.

The mean BMI reported in most RA studies (ranging from 26.5-28.2kg/m^2) (Gordon *et al.*, 2002b, Saravana and Gillott, 2004) is similar to that of the general population in the UK (about 27.1kg/m^2) (The Information Centre NHS, 2005). Prevalence of overweight and obesity, as assessed by the general BMI cut-off points, in RA varies according to geographic area. A world-wide study identified 18% (Naranjo *et al.*, 2008) of RA patients as obese and a UK based study found 31% (Armstrong *et al.*, 2006). However, in both studies >60% of RA patients

exhibited BMI above the desired levels (i.e. 25kg/m^2). Results from other studies lie within this range indicating that overweight and obesity, even when assessed based on the general BMI cut-offs, is highly prevalent among patients with RA (Gordon *et al.*, 2002a).

2.3.2 Obesity as a Risk Factor for the Development of Rheumatoid Arthritis

Due to the long-established close association of obesity with osteoarthritis several authors have investigated the potential association of obesity with the risk for the development of RA. In the first studies on this matter, obesity in females was associated with an odds ratio (OR) for RA of 1.4 [95% confidence intervals (CI) = 1.0-2.0] (Voigt *et al.*, 1994); in a larger sample including both genders the association of obesity with RA was even greater (OR= 3.74, 95% CI: 1.14-12.27) (Symmons *et al.*, 1997). Newer studies however, have failed to produce consistent results. Obesity has been found not to confer any risk for the development of RA whatsoever (Bartfai *et al.*, 2007), and to influence its development (OR= 3.45; 1.73-6.87) only in a small proportion of RA patients who test negative for anti-cyclic citrullinated peptide (anti-CCP) antibodies (Pedersen *et al.*, 2006). Even though the reason for this discrepancy is not discussed by the authors it seems that methodological differences and tight standardisation for possible confounders in recent studies eliminate the previous positive findings for the association of obesity with RA development.

2.3.3 Obesity and Disease Activity

Adipose tissue, initially considered to be simply an energy reservoir, is now recognised as a metabolically active tissue. It secretes a number of bioactive proteins called adipokines or adipocytokines, including TNFα and IL-6 (Pi-Sunyer, 2006). Both molecules are implicated in the development and progression of RA (Buch and Emery, 2002).This could potentially result in more active disease in obese RA patients.

Studies in patients with early RA, of up to 3 years duration, surprisingly suggest that obesity may protect against joint damage (Kaufmann *et al.*, 2003, Westhoff *et al.*, 2007, van der Helm-van Mil *et al.*, 2008). On a similar line, changes in body weight over 1-year do not correlate with changes in disease activity during the same period (Morgan *et al.*, 1997). The protective effect seems to be present before the diagnosis of the disease, with overweight or obese RA patients exhibiting less joint damage than their normal weight counterparts at the time of diagnosis (Westhoff *et al.*, 2007), and to continue during the first few years of the disease, with joint damage progressing less rapidly in obese than in normal-weight RA patients (Kaufmann *et al.*, 2003, Westhoff *et al.*, 2007, van der Helm-van Mil *et al.*, 2008). However, it is not entirely clear whether this is solely an effect of increased weight, for example through increased mechanical loading stimulating bone synthesis (Tremollieres *et al.*, 1993), or also a reflection of joint damage at the time of first diagnosis.

In contrast to these observations, studies in unselected (for disease duration) RA patients suggest that obesity leads to worse quality of life (Garcia-Poma *et al.*, 2007) and a narrowing in the joint space of the knees (Hollingworth *et al.*, 1982); indicating that its potential protective effects in early RA are diminished

or reversed later on in the course of the disease. Interestingly, all studies suggest a deleterious effect of low BMI in disease duration and quality of life. Most likely, significantly reduced BMI is the result rather than the cause of highly active disease over many years (Roubenoff *et al.*, 1994). However, to date no studies have investigated the associations of obesity with disease activity and joint damage in patients with established RA.

2.3.4 Obesity and Risk for Cardiovascular Disease

Obesity is a well established and independent risk factor for CVD (Bray and Bellanger, 2006). It is also considered to be the underlying cause of many other CVD risk factors such as hypertension, dyslipidaemia, and insulin resistance (Grundy *et al.*, 2004) and a potent contributor to the inflammatory state of atherosclerosis (Berg and Scherer, 2005). In RA several studies have investigated the effects of obesity on CVD risk and their results are conflicting. Obese RA patients are likely to exhibit classical CVD risk factors (Armstrong *et al.*, 2006) and obesity significantly contributes to the increased 10-year CVD risk event probability of RA patients (Kremers *et al.*, 2008) as calculated with the Framingham formula (Anderson *et al.*, 1991). However, in patients with low BMI (<18.5kg/m^2) 10-year CVD risk event probability was even higher (Kremer, 2001). In a longitudinal study of RA patients results were different for high but similar for low BMI. Patients with a BMI of >30kg/m^2 exhibited reduced all-cause mortality (Escalante *et al.*, 2005). As BMI decreased, mortality increased and patients with a low BMI (<20kg/m^2) exhibited significantly increased rates of mortality. On the same line are data from the QUEST-RA study, a large multinational study, that found no associations between obesity and CVD morbidity (Naranjo *et al.*, 2008).

Thus, there is evidence to suggest the presence of reverse epidemiology. This refers to paradoxical and counterintuitive epidemiologic associations between survival outcomes and traditional CVD risk factors such as obesity (Horwich and Fonarow, 2007). In diseases associated with accelerated loss of FFM, over-nutrition (a long-term killer) might protect against the significant health consequences of reduced FFM while under-nutrition might enhance muscle wasting and through it accelerate mortality (Kalantar-Zadeh *et al.*, 2007).

However, before we jump to conclusions some significant limitations of these studies need to be noted. In the study of Escalante and colleagues (2005) the protective effect of BMI was present only when the erythrocyte sedimentation rate (ESR), an indicator or disease activity, was low. This would suggest a more significant influence of disease activity rather than obesity in the CVD of RA patients. This could also bias the results as a low disease activity associates with higher levels of FFM (Roubenoff *et al.*, 1994). Furthermore, lower disease activity is usually achieved through medication. Stable and long exposure to medication, that leads to effective control of inflammation, is also associated with reduced CVD risk (Kremers *et al.*, 2008). Finally, obesity on its own is a determinant for CVD treatment. Obese individuals are more likely to receive medication for CVD risk factors modification than their non-obese counterparts (Narbro *et al.*, 2002, Chobanian *et al.*, 2003), thus CVD medication should always be included in studies investigating the effects of obesity on CVD risk in RA patients as well as in the general population. Based on the above, the associations between obesity and CVD risk in RA are far from clarified.

2.3.5 Control of Obesity

The control of obesity in RA has received minimal scientific attention. This search of the literature was able to identify only two studies aimed at reducing body weight of patients with RA. The first used caloric restriction over a period of 12 weeks, in combination with a protein-rich diet and low-intensity physical activity. This intervention resulted in a small weight loss of 2.7kg. However, most of the weight loss (1.7kg) came form a reduction in FFM (Heitmann *et al.*, 1994). The second study had a similar design (i.e. 12 weeks of caloric restriction with protein supplementation) but utilised physical activity of moderate intensity. The results indicated a 4.5kg reduction in body weight with only a minimal loss of FFM (>1kg) (Engelhart *et al.*, 1996). These findings are in line with recent data of studies investigating ways to reverse rheumatoid cachexia. Marcora *et al.* (2005a) investigated the effects of 12 weeks of protein supplementation on lean body mass of RA patients. They suggested that increased protein intake is able to reverse rheumatoid cachexia and significantly increase lean body mass in such patients. However, they were not able to find any changes in body weight or body fat. The same group also investigated the effects of a 12-week resistance exercise intervention on components of rheumatoid cachexia. Lean body mass was again increased as a result of the exercise; interestingly, and even though that was not one of the primary objectives of the trial, total body fat was marginally (1.1%) but significantly reduced and trunkal fat showed a tendency to reduce (Marcora *et al.*, 2005b).

2.3.6 Conclusions

From this short review of the limited literature on obesity in RA, several questions arise. First of all, the definition of overweight and obesity used in RA (i.e. that of the WHO designed for the general population) might not be accurate for RA patients. Secondly, the associations of obesity with disease activity and CVD are conflicting and the methodology used in most studies has several potential limitations. Also, ways to counteract obesity in RA need to be developed and tested, always taking into account the wasting state of these patients. However, in order to do that, the potential causes of obesity in RA need to be identified.

Chapter 3: Aims and Hypotheses

The principal aims of the present project were to:

1. a) Investigate whether BMI and BF differ between patients with RA, osteoarthritis (OA), or healthy controls and within RA patients according to disease state (e.g. active vs. inactive, early vs. established disease); b) if necessary, develop RA specific measures of adiposity; c) assess the associations of BMI and BF with RA characteristics in patients with well-established disease.
2. Quantify the associations of BMI with classical CVD risk factors in a large sample of RA patients.
3. Identify subgroups of the RA population that exhibit phenotypic characteristics different from those expected for their BMI class, and investigate the health effects of obesity in these subgroups.
4. Identify factors that might reduce or increase BMI and BF in RA.

For each of the above aims the following hypotheses were made:

1. a) RA patients might have increased BF compared to both patients with OA and healthy controls of the same BMI; b) Overall BMI, and the prevalence of overweight and obesity, in RA patients might also be increased; c) RA patients with highly active and/or long-standing disease might exhibit even higher BF but not BMI levels.
2. Most likely, RA specific measures of adiposity will be required. These would have to take into account the muscle wasting associated with the disease.

3. Due to increased BF, obese individuals are likely to have higher levels of inflammation compared to normal-weight individuals. This might also affect joint damage.
4. Increasing BMI and BF are expected to associate with worse CVD profile. Most likely, use of CVD medication will further increase this association.
5. Some normal-weight patients are expected to exhibit increased CVD risk while some obese patients reduced. This might also affect their inflammatory status.
6. The factor most likely to influence BMI and BF of RA patients is inflammation and its control through medication. To a lesser extent, physical activity and diet might also affect these factors.

Chapter 4: Methodology

4.1 Participants

For the purposes of this project a total of 1167 volunteers were assessed. Of them 1042 were RA patients, 43 were patients with OA, and the remaining 82 healthy controls. In the first study, data derived from 516 RA patients were compared against all 43 OA patients and all 82 controls. In studies two, three, four and five data from a different set of 400 patients with RA were used. Data from the remaining 126 RA patients were used in the sixth and final study. All RA patients met retrospective application of the 1987 American College of Rheumatology (ACR) classification criteria for RA (Arnett *et al.*, 1988). Individuals with diagnosed OA of the hip (Altman *et al.*, 1991) or knee (Altman *et al.*, 1986) were included in the OA groups, and individuals who by self-report did not have any known clinical conditions and were taking no medication were included in the healthy control group. For the RA and OA groups, consecutive patients attending routine rheumatology or orthopaedic outpatient clinics at the Dudley Group of Hospitals NHS Trust, UK, were invited to participate. Staff from the same hospital as well as the University of Wolverhampton, Walsall Campus, UK, formed the healthy control group. All participants were given verbal and written information about the project and signed an informed consent according to the declaration of Helsinki (World Medical Association, 2000). Due to the specific requirements of each study, further details about the participants are presented in the methods section of the respective studies.

4.2 Procedures

Participants were asked to visit the testing venue (Rheumatology Unit of the hospital) early in the morning following a 12hour overnight fast. They were also instructed to keep their physical activity levels to a minimum on the day prior to the assessment. All volunteers were subjected to the same data collection procedures overseen by the same trained investigators. Upon arrival, participants' anthropometric characteristics and body composition were assessed. Then, participants were asked to rest for 10 minutes on a bed placed in a quiet, thermoregulated room (21-23^{o}C) with dimmed lights. Conventional CVD risk factors and RA disease characteristics were assessed thereafter. A blood sample was also drawn.

For the participants of the final study, a physical activity questionnaire was completed following the above assessments. Also a food diary was given to the patients with instructions on how and when to fill it in.

4.3 Assessments

All assessments are described here in full detail. However, they are also presented in the studies to help the reader. The information presented in the studies is only a summary of the individual assessments.

4.3.1 Anthropometry and Body Composition

Standing height was measured to the nearest 0.5cm on a Seca 214 Road Rod portable stadiometer (Seca gmbh & co. kg., Hamburg, Germany). Body composition was assessed by BIA, using a Tanita BC-418 MA Segmental Body Composition Analyzer, which incorporates 8 tactile electrodes (Tanita Corporation, Tokyo, Japan). This apparatus, measures total body mass (i.e. body

weight) to the nearest 0.1kg and assesses body composition in terms of percentage BF, fat mass, FFM, and total body water, as well as fat distribution in different body segments (abdominal and peripheral fat). The specific device has a standard error of <3% when standard procedures are followed (Tanita, 2002). These procedures aim at reducing alterations in hydration status. As stated in the relative section of the literature review (2.2.2.3, p. 40-41), BIA essentially assesses water and electrolyte content of different parts of the body (Bolanowski and Nilsson, 2001). Thus, patients were asked to refrain from excessive fluid consumption the day before the assessment, and they were instructed to drink two pints of water in the morning prior (1-2 hours) to the assessment. After initial manual entry of their demographic details, participants stood bare-footed on the analyzer and held the handgrips provided until the apparatus printed the results. BMI (kg/m^2) was calculated on the basis of measured height and weight. Waist circumference was measured using a Seca 200 Circumference measuring tape (Seca gmbh & co. kg., Hamburg, Germany). Cut-offs for increased waist circumference were >102cm for males or >88cm for females (Lean *et al.*, 1995).

4.3.2 Assessments of Aspects of Rheumatoid Arthritis

4.3.2.1 Erythrocyte Sedimentation Rate

ESR is an additional measure of disease activity; however it may reflect longer-term effects of the disease than CRP. ESR was measured using a Starrsed compact (Mechatronics BV, Netherlands). A total of 10ml of undiluted blood, anti-coagulated with EDTA is inserted in a vertical tube. The sedimentation (in millimetres) of the red blood cells within one hour gives the value of ESR.

4.3.2.2 C-reactive Protein

CRP is a measure of acute RA activity closely associated with inflammation. It was measured with a Vitros® 5.1 FS chemistry system (Johnson and Johnson Inc., Langhorne, PA, USA). This system uses a slide which is a multi-layered analytical element coated on a polyester support. A drop of the patient's serum or plasma is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. Measurement is based on an enzymatic heterogeneous, sandwich immunoassay format. In this format a derivative of phosphorylcholine is covalently bound to polystyrene polymer beads and the presence of calcium serves as a capture agent (Park *et al.*, 2004). Monoclonal anti-CRP antibody labelled with horseradish peroxidase serves as a signal generator. CRP in the blood sample binds to the phosphorylcholine-linked capture beads and the anti-CRP antibody labelled with horseradish peroxidase to form an insoluble sandwich complex in the first incubation. This is subsequently washed with a specific liquid which also provides the hydrogen peroxide for the enzyme mediated oxidation of leuco-dye. The reflection density of the dye is measured after a second wash giving a reflection density directly proportional to the concentration of CRP in the sample.

4.3.2.3 Disease Activity

The disease activity score 28 (DAS; Appendix 1) was used to assess disease activity, being a composite assessment of acute and longer-term disease activity. It consists of three components (Prevoo *et al.*, 1995). Initially pain and swelling of 28 joints of the patients (i.e. shoulders, elbows, wrists, hands and knees) are assessed by gently squeezing them. Then the patient grades his/her overall health during the last week on a visual analogue scale from zero (best ever

health) to 100 (worst ever health). The values from these assessments together with current ESR are included in a formula which gives the DAS.

4.3.2.4 Physical Function

Physical function is the reflection of chronic disease activity and associates with the long-term severity of the disease. It was assessed using the Anglicised version of the 40-item Stanford Health Assessment Questionnaire (HAQ; Appendix 2) (Kirwan and Reeback, 1986). Participants rate their ability (over the past week) to carry out 20 activities within eight aspects of daily living (dressing/grooming, rising, eating, walking, hygiene, reach grip and errands/tasks) on a four-point scale from 'without any difficulty' to 'unable to do'. For each aspect patients also respond whether they receive assistance from people or use specific devices. The HAQ is internally consistent ($\alpha \geq .89$) and has excellent pre- to post-physician visit temporal stability ($r = .99$). Physiotherapists' ratings have excellent agreement with RA patients' ratings (Treharne *et al.*, 2006, Witney *et al.*, 2006).

4.3.2.5 Cytokines

Pro-inflammatory cytokines (i.e. IL-1, IL-6 and TNFα) were assessed in stored serum in batches. As soon as the blood sample was collected, it was centrifuged at 4000rpm for 10 minutes (relative centrifuge force = 1650 g). The supernatant was collected in aliquots and frozen immediately at -70^{o}C. Specimens were analysed, in groups of 48 (analyses were duplicated to assure accuracy of the results), using multi-analyte Biochip Array Technology (Evidence analyzer, Randox, USA).

4.3.2.6 Rheumatoid Factor and Anti-cyclic Citrullinated Peptide Antibodies

Screening for the presence of rheumatoid factor (RF) and anti-CCP antibodies is carried out by a manual particle agglutination method (MAST diagnostics, Merseyside, UK). The test for RF and anti-CCP is based upon the immunological reaction between their concentration in serum and the corresponding human antibodies which are coated onto polysterene latex particles. If serum containing either of the two is mixed with the latex, a resulting agglutination will be observed. Positive tests are quantified using an Enzyme-Linked Immuno-Sorbent Assay (i.e. ELISA) test. Diluted serum is added to wells coated in purified antigen; the antibodies bind to the antigen. This is incubated at room temperature and unbound material is rinsed away. Then horseradish peroxidase conjugated anti-IgG monoclonal antibody is added and binds to immobilised antibodies. After a second incubation and rinse cycle tetra-methyl benzidine substrate is added to each well. The presence of the antigen-antibody-conjugate complex turns the substrate a dark blue colour. Addition of a stop solution turns it yellow; the colour intensity measured by photospectrometry is proportional to the concentration of the antibodies in the original sample.

4.3.2.7 Other Assessments of Rheumatoid Arthritis

Review of patients' clinical notes revealed duration of RA symptoms, current medication as well as joint surgery. Presence of joint erosions was assessed on x-rays of hands and wrists by two independent rheumatologists; in case of disagreement, x-rays were jointly reviewed and a consensus opinion reached.

4.3.3 Assessments of Cardiovascular Parameters

4.3.3.1 Blood Pressure

Blood pressure (BP) was assessed three times on the brachial artery, using a Datascope Accutorr Plus (Datascope, Montvale, NJ, USA), while the patient was resting on a supine position. The average of the three measurements is reported herein. Patients with systolic BP≥140 and/or diastolic BP≥90 and/or receiving antihypertensive medication, were characterised as hypertensive (Williams *et al.*, 2004).

4.3.3.2 Blood Lipids

Triglycerides, total cholesterol, and high density lipoprotein (HDL) were measured using the same Vitros® 5.1 FS chemistry system (Johnson and Johnson Inc., Langhorne, PA, USA) as for the assessment of CRP. The same chip was used as well; however, instead of an enzymatic heterogeneous, this time a dye that binds to the chemical to be measured from the sample was used. This results in a shift in wavelength of the reflectance maximum of the free dye. The colour complex that forms is measured by reflectance spectrophotometry. The amount of chemically-bound dye is proportional to the concentration of the chemical being measured in the sample.

In the case of low density lipoprotein (LDL), the kit was a dual chamber package instead of a chip that contains two different reagents. The first reagent selectively eliminates non-LDL cholesterol. The second reagent dissociates cholesterol from cholesterol esters and proteins and promotes the reaction with cholesterol esterase and cholesteroloxidase. Hydrogen peroxide is a by-product, which is then dyed and measured spectrophotometrically at 600nm.

Patients with increased levels of triglycerides (>1.7mmol/L), total cholesterol (>6.2mmol/L), LDL (>4.13mmol/L), decreased levels of HDL (<1.03mmol/L) or receiving lipid lowering therapy were characterized as dyslipidaemic (Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults, 2001).

4.3.3.3 Insulin Resistance

For the assessment of blood glucose, the same Vitros® 5.1 FS chemistry system (Johnson and Johnson Inc., Langhorne, PA, USA) as above was used and the same procedure as with cholesterol (not LDL) was followed. Insulin was estimated from serum stored at -20ºC. The method of detection involved a solid phase two-site chemi-luminescence immunometric assay. The Immunolite 2000 insulin was used on the Immulite 2000 Analyser (Siemens Healthcare Diagnostics, Deerfield, IL, USA). Insulin resistance (IR) was evaluated from fasting glucose and insulin using the Homeostasis Model Assessment of insulin resistance (HOMA) (Radikova, 2003) and the Quantitative Insulin sensitivity Check Index (QUICKI) (Katz *et al.*, 2000), and was defined as HOMA ≥ 2.5, QUICKI ≤ 0.333, presence of diabetes mellitus or use of anti-diabetic medication.

4.3.3.4 Composite Assessments of Cardiovascular Risk

The metabolic syndrome, a constellation of several risk factors was assessed according to the NCEP ATP III criteria (Grundy *et al.*, 2004). Specifically, patients were diagnosed with the MetS if they presented three of the following: Increased waist circumference, high BP, high triglycerides, low HDL and/or fasting glucose above 110mg/dL. Smoking status was also noted and the Framingham 10-year CVD event probability score was calculated (Anderson *et al.*, 1991).

4.3.4 Lifestyle Assessments

4.3.4.1 Physical Activity

The long version of the self-administered international physical activity questionnaire (IPAQ; Appenix 3) was utilized to record physical activity. This questionnaire is divided into five parts requesting information about the physical activities (job-related, transportation, housework, leisure time and time spent sitting) that the participants had undertaken over the previous seven days. The same nurse always helped the patients to fill in the questionnaire. The IPAQ has been extensively used for research purposes, and its validity and reliability have been assessed in 12 countries, including the UK (Craig *et al.*, 2003).

4.3.4.2 Energy Intake

Energy intake was assessed with the use of a food diary (Appendix 4). This method requires the patients to keep a written record of anything they eat or drink, including portion sizes (Macdiarmid and Blundell, 1998). It is a relatively easy procedure that is considered valid and reliable, however obese individuals are known to underreport energy intake (Livingstone, 1995, Macdiarmid and Blundell, 1998). In the present project, a simple three-day food diary was distributed to the participants. Relevant verbal and written instructions were given at the day of the assessment. They were asked to write in detail about all the food and drink they consumed over three non-consecutive days (including one weekend day) during the week following the assessment. They were also instructed to follow their normal diet. Total daily energy intake and percentage carbohydrate, fat and protein intake were calculated using diet analysis software (Recipe Calc 4.0).

4.4 Data Management and Analyses

For all studies data were inserted in a purpose-designed spreadsheet (Microsoft Excel 2003) and audited for accuracy weekly. They were exported for analysis to The Statistical Package for Social Sciences version 15.0 (SPSS Inc. Chicago, IL, USA). The Kolmogorov-Smirnov test of normality assessed dispersion of the variables. Due to the complexity of the project several different analyses were used. These are described in detail in the respective studies.

Chapter 5: Studies

5.1 Redefining Overweight and Obesity in Rheumatoid Arthritis

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5.1.1 Introduction

Excess BF is a prominent health hazard (Van Pelt *et al.*, 1997) significantly contributing to the development of cardiovascular disease (CVD) (Ross, 1999). About two-thirds of patients who have had a myocardial infraction (MI) exhibit increased body weight (Romero-Corral *et al.*, 2006). Obesity increases the risk of coronary heart disease (CHD) through a number of different pathophysiological pathways, including insulin resistance, type 2 diabetes, hypertension and dyslipidaemia (Krauss *et al.*, 1998, Pi-Sunyer, 2002).

Assessments for overweight or obesity include the calculation of BMI (in kg/m^2) or more accurate estimations of relative adiposity (BF) through a number of techniques (e.g., skinfold thickness, hydrostatic weighing, and bioelectrical impedance) (Nevill *et al.*, 2004). BF estimations require sophisticated equipment and trained personnel, whereas BMI is easy to obtain and is widely used in the routine clinical setting.

In the general population, BMI of <25, 25-30, and >30kg/m2 indicate healthy, overweight, and obese individuals and associate with low, medium, and high CVD risk, respectively (WHO, 1998, Calle *et al.*, 1999). However, BMI is only a proxy of body fat (Wellens *et al.*, 1996), and over recent years its validity has been questioned (Manson *et al.*, 1995, Willett *et al.*, 1995, Blew *et al.*, 2002, Nevill *et al.*, 2004, WHO, 2004, Romero-Corral *et al.*, 2006). Overweight as defined by

BMI of >25, has poor specificity in detecting excess body fat in healthy men and women of all ages (Wellens *et al.*, 1996) as well as in patients with coronary heart disease (Romero-Corral *et al.*, 2006). In specific sub-populations, such as Indian-Asian (WHO, 2004), women (Manson *et al.*, 1995, Willett *et al.*, 1995, Blew *et al.*, 2002), and large size athletes (Nevill *et al.*, 2004), new BMI cut-off points have been suggested, that optimally reflect BF and may better predict CVD risk.

The weakness of BMI is that it does not distinguish between lean-body mass and fat mass. Consequently people of similar stature and weight, but different muscle content, will have the same BMI but different BF levels. This tends to be more evident in individuals with low BMI levels (Wellens *et al.*, 1996). Such limitations of the BMI may explain the better cardiovascular outcomes observed in overweight and mildly obese patients with established CHD as compared to their normal-weight counterparts, who may have proportionately more BF (Romero-Corral *et al.*, 2006). Therefore, although it is well established that CHD risk increases with advancing BMI levels (WHO, 1998), global cut-off points may be misleading for several populations.

Central obesity poses a great risk for cardiovascular disease. Regional fat distribution, as measured by waist-to-hip ratio, has been proposed as a more accurate predictor of CHD risk than BMI (Hsieh and Yoshinaga, 1995, Rimm *et al.*, 1995). Indeed, it has been suggested that obesity should be redefined based on waist-to-hip ratio instead of BMI, since waist-to-hip ratio is significantly associated with MI risk in most ethnic groups (Yusuf *et al.*, 2005). However, its predictive strength can be negatively affected by gender and overall body weight (Li *et al.*, 2006), in a way that pear- shaped or obese individuals might have optimal waist-to-hip ratio but increased overall body weight. More research is

necessary to identify the optimal definition of obesity as a predictor for CHD in the general population and specific sub-groups (Bray, 2004).

Patients with RA have an increased risk for CHD events (Kitas and Erb, 2003). RA is a chronic inflammatory disease which affects predominantly synovial joints, causing pain, swelling, stiffness and eventually irreversible damage and deformity, all of which may lead to significant reduction in physical activity. RA associates with increased mortality particularly from CHD (Kitas and Erb, 2003), most probably due to accelerated atherogenesis secondary to the metabolic and vascular effects of systemic inflammation (Stevens *et al.*, 2005). Nearly two thirds of all individuals with RA experience involuntary loss of fat-free mass and progressively increased fat mass in the presence of stable or even slightly decreased weight, a condition referred to as rheumatoid cachexia (Rall and Roubenoff, 2004). The exact mechanisms causing rheumatoid cachexia remain undetermined, but muscle loss due to systemic inflammation and reduced physical activity may both contribute (Metsios *et al.*, 2006).

We hypothesised that for a given BMI, RA patients exhibit significantly higher proportions of fat mass than healthy individuals, or even than patients with movement restriction due to a non-inflammatory arthritis, such as OA. The possible consequences of this, in the context of the increased CVD mortality in RA, are obvious. In the present study we aimed to investigate whether BMI and BF differ according to arthritic disease (OA vs. RA) and within RA according to disease state (e.g. active vs. inactive, early vs. established disease). We also developed and validated RA-specific BMI cut-off levels and algorithms to calculate BF from BMI.

5.1.2 Methods

Participants

Consecutive patients attending routine rheumatology or orthopaedic outpatient clinics at the Dudley Group of Hospitals NHS Trust, UK, and healthy controls (Hospital and University staff) were invited to participate. The study had Local Research Ethics Committee approval by the Dudley Ethics Committee, and all volunteers provided informed consent. The observation group (n=299) included 174 volunteers with RA [1987 revised ACR criteria (Arnett *et al.*, 1988)], 43 with OA of the hip (Altman *et al.*, 1991) or knee (Altman *et al.*, 1986), and 82 healthy controls (HC; individuals who by self-report did not have any known clinical conditions and were taking no medication). The validation group (n=342) consisted of RA patients only. Demographic and disease characteristics from all subjects appear in Table 9.

Assessments

Standing height was measured to the nearest 0.5cm on a Seca 214 Road Rod portable stadiometer (Seca gmbh & co. kg., Hamburg, Germany). Body weight was measured to the nearest 0.1kg. Body composition was assessed by Bioelectrical Impedance, using a Tanita BC-418 MA Segmental Body Composition Analyzer, which incorporates 8 tactile electrodes (Tanita Corporation, Tokyo, Japan). BMI (kg/m2) was calculated on the basis of measured height and weight. In RA patients, contemporary serological inflammation and clinical disease activity were assessed by the ESR, CRP and the DAS (Prevoo *et al.*, 1995). Disease duration was recorded from review of the patients' hospital notes.

Data management and analysis

Data were inserted in a purpose-designed spreadsheet (Microsoft Excel 2003) and audited for accuracy weekly. They were exported for analysis to The Statistical Package for Social Sciences version 11.0 (SPSS Inc. Chicago, IL, USA). Preliminary evaluation of the variables using a Kolmogorov-Smirnov test of normality revealed that none of them required logarithmic transformation to reach normality. Means (sd) were calculated for all variables.

The method of analysis was to define either BMI or BF as the dependent variable and then to incorporate all other known parameters thought to influence these measures of adiposity as either factors, in analysis of variance (ANOVA), or factors with covariates in analysis of co-variance (ANCOVA). Factors included gender and disease status (RA, OA and HC) while age, disease activity and duration, and serological inflammation were entered as continuous covariates. The initial ANCOVA analysis incorporated all these factors and covariates, but only those found to be significant were subsequently retained and reported in the prediction equation model below.

Table 9: Demographic and disease characteristics of all volunteers for study one and results of ANOVA [mean (sd)]

	Observation group						Validation Group	
	MALE (n= 110)			FEMALE (n= 189)			Male	Female
	RA	OA	HC	RA	OA	HC	RA	RA
N	56	15	39	118	28	43	99	243
Age (years)	60.6 (11.8)**	56.7(13.3)*	45.1 (13.3)	59.6 (12.2)** †	52.8 (12.5)*	46.8 (11.5)	62.1 (11.6)	61.7 (11.9)
Height (cm)	173.6 (7)*	171.3(6.7)*	177.3 (6.7)	159.1 (6.5)**	161 (5)	163.6 (6.9)	174 (6.8)	160,4 (6.7)
Weight (kg)	83.6 (13.3)	78.4 (14.8)	80.9 (11.4)	68.6 (15)	70.8 (16.5)	68.1 (16.3)	82.7 (15.8)	70.2 (14.4)
BMI (kg/m^2)	27.7 (4.3)*	26.8 (4.7)	25.7 (3)	26.9 (5.7)	27.2 (5.7)	25.4 (5.5)	27.3 (4.4)	27.3 (5.3)
BF (%)	28.7 (7.7)**	24.8 (7.9)*	19.2 (5.2)	38.3 (7.3)** †	35.2 (8.5)	32.1 (8.2)	27 (6.4)	38.3 (7.1)
Trunkal Fat (%)	30.5 (8)**	26.6 (8.9)*	21.4 (6)	35.7 (8.6)** †	31.6 (9.6)	29.1 (8.7)	27.4 (7.7)§	35.4 (8.1)
DAS	4.2 (1.2)			4.3 (1.4)			4.1 (1.4)	4.3 (1.4)
ESR (mm/h)	23.2 (18.5)			26 (22.1)			25.3 (21.5)	30 (26.3)
CRP (mg/L)	15.6 (15)			15.8 (14.9)			16.8 (18.6)	17.6 (23.6)
Disease Duration (years)	11.4 (10.2)			11.3 (9.9)			12.5 (11)	13.2 (11)

* Significant difference compared to HC ($p<0.05$) ** Significant difference compared to HC ($p<0.001$)
† Significant difference compared to OA ($p<0.05$) § Significant difference compared to experimental RA group ($p<0.001$)

Within the RA population of the observation group, correlations of disease activity (DAS, ESR, and CRP) and disease duration with BMI and BF were obtained for each gender. RA patients were also sub-grouped according to their clinical disease activity [DAS remission < 2.6, mild 2.7 – 3.2, moderate 3.3 – 5.1, high > 5.1 (Prevoo *et al.*, 1995)], serological inflammation [ESR (Brigden, 1999) and CRP (Black *et al.*, 2004)], disease duration (early <3 years, established 3-10 years, longstanding >10 years), rheumatoid factor positivity (ever), or corticosteroid administration (yes/no ever): differences between these sub-groups in relation to BMI and BF were assessed using ANCOVA (Table 9). The level of significance was set at $p<0.05$.

The external validity of the predictive model was tested with the Limits of Agreement (LIMAG) method (Bland and Altman, 1986) against BF of the validation group. The Limits of Agreement were obtained as follows:

We calculated the mean (d) and the standard deviation (s) of the differences that indicate the level of bias and the random variation between the two measures of BF (i.e. the predicted BF and measured BF of the validation group, respectively). Provided the differences are normally distributed, the 95% 'Limits of Agreement' are given by: $d \pm (1.96 \times s)$.

Bland and Altman (Bland and Altman, 1986) argue that, provided that differences within these limits are not clinically important, the two measurement methods can be used interchangeably.

5.1.3 Results

Observation group

Within the RA population of the observation group, no significant correlations were found between DAS, ESR, CRP, disease duration and BMI or BF. Similarly, when RA patients were grouped according to these variables as well as rheumatoid factor positivity and corticosteroid use, no significant differences for BMI and BF were observed ($p>0.05$ in all cases, see Table 10).

Between the different disease groups, one-way ANOVA revealed significant differences in BMI ($p<0.05$) and BF ($p<0.001$; Table 9): RA males had higher BMI and BF (including trunkal fat) than HC males, and RA females had higher BF than HC females, even though their BMI did not differ significantly. ANCOVA revealed that BMI differences between the groups were mainly due to the significant effect of the covariate age ($F_{1,\ 294} = 5.10$, $p <0.05$) and not due to disease ($F_{2,\ 294} = 1.00$, $p >0.05$), gender ($F_{1,\ 294} = 0.59$, $p >0.05$), or their interactions.

ANCOVA also revealed that RA and OA patients exhibited lower BMI levels than their HC for a given BF. However, differences were only significant for the RA patients [RA:-1.826 kg/m2 ($p<0.001$); OA: -0.352 kg/m2 ($p>0.05$)]. BMI was significantly ($p<0.001$) predicted by age, disease, gender and BF ($R^2= 0.58$).

Table 10: BMI and BF of RA patients (observation group) according to categorisation based on their disease characteristics.

Disease Characteristics	Categories	BMI		BF	
		Male	**Female**	**Male**	**Female**
DAS	Remission (<2.6)	27.2 (3.46)	27.2 (5.6)	26.5 (7.6)	39.5 (6.7)
	Mild (2.7- 3.2)	28 (4.3)	27.3 (4.6)	28 (6)	39.3 (6.6)
	Moderate (3.3 -5.1)	27.8 (4.5)	27 (5.3)	27.4 (6.8)	37.3 (7.7)
	High (>5.1)	25.3 (5.5)	27.3 (5.5)	26.1 (5.6)	37.7 (7.2)
ESR (mm/h)	Normal*	27.9 (4.4)	26.9 (4.8)	27.1 (7.2)	38.3 (6.3)
	High	26.4 (4.6)	27.6 (6.1)	26.7 (5.9)	37.6 (8.9)
CRP (mg/ L)	Low (<3)	26.5 (2.4)	28.3 (6.2)	25.9 (5.4)	38.5 (8.7)
	Normal (3 – 8)	27.8 (4.7)	26.5 (4.7)	26.7 (8)	37.6 (6.6)
	High (>8)	26.9 (4.6)	27.6 (5.7)	27.3 (5.8)	38.3 (7.9)
Disease Duration (years)	Early (<3)	26.4 (5)	26.1 (5)	26.4 (7.9)	37.9 (8.3)
	Established (3-10)	28.8 (4.1)	27.8 (5.7)	27.8 (6.3)	38.2 (7.5)
	Longstanding (>10)	26.8 (4.4)	27.1 (5.1)	27.7 (5.7)	38.8 (6.7)
Rheumatoid Factor (%)	Positive	26.6 (3.6)	27.2 (5.7)	25.1 (6.7)	38.3 (7.3)
	Negative	27.5 (5)	27.1 (5.1)	27.7 (6.4)	37.9 (7.3)
Corticosteroid Administration (%)	Yes	27.1 (4.4)	27.3 (5.3)	26.2 (6)	38.1 (7.4)
	No	24.5 (4.9)	26.7 (5.3)	27.8 (7.3)	37.7 (7)

For all differences between groups: p>0.05

* Normal ESR: < 50 years: male <15, female <20
> 50 years: male <20, female <30

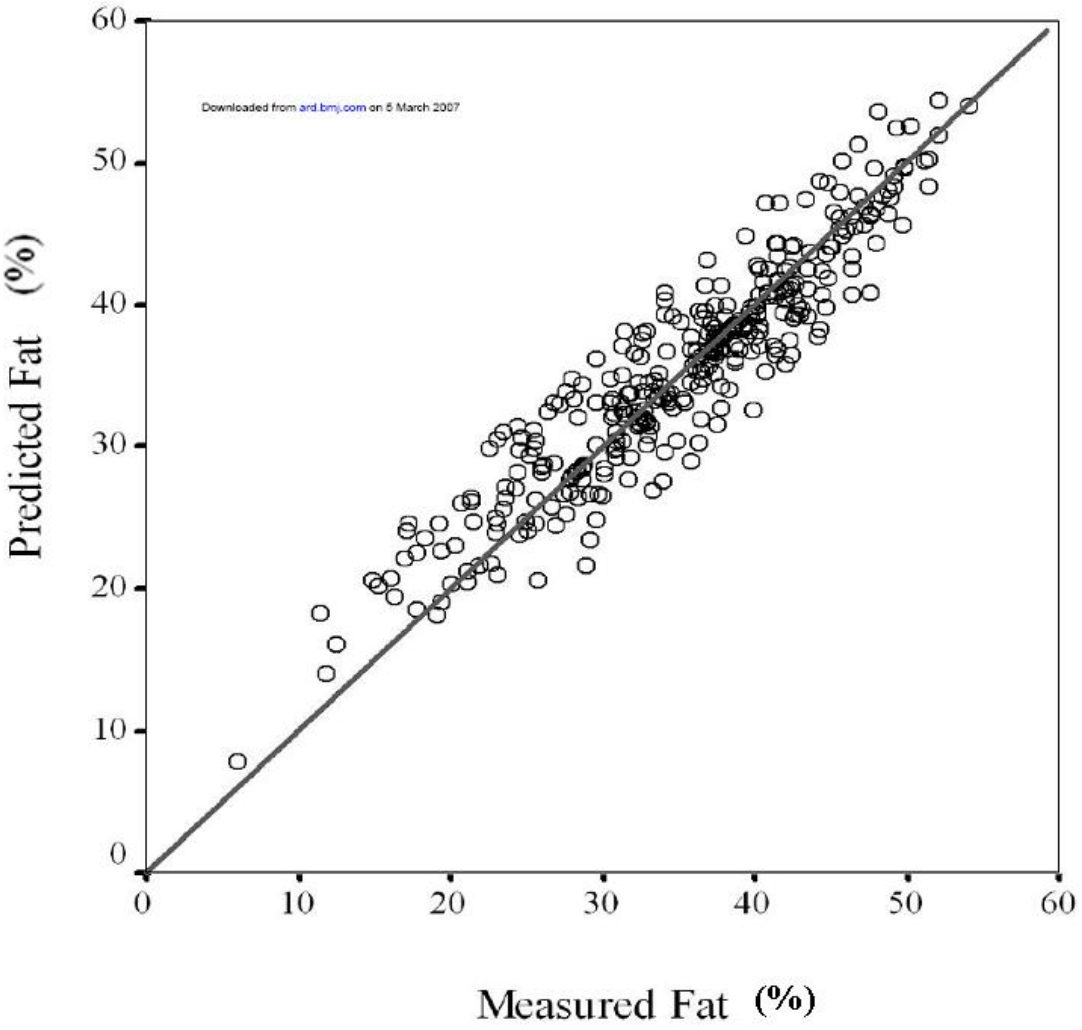
When BF was adopted as the dependent variable, ANCOVA identified significant differences between disease groups ($F_{2\text{-}293}$= 18.70, p<0.001) and gender ($F_{1\text{-}293}$= 380.90, p<0.001) together with a significant covariate, age ($F_{1\text{-}293}$= 22.43, p<0.001). The contribution of BMI as a covariate in this analysis was also significant ($F_{1\text{-}293}$= 370.74, p<0.001). For a given BMI, RA patients exhibited significantly increased levels of BF ($F_{2\text{-}293}$= 4.273, p<0.001) compared to healthy controls. The difference for OA patients was non-significant ($F_{2\text{-}293}$= 1.648, p>0.05). The variation of BF was predicted by age, gender, BMI, and disease type (R^2= 0.769, p<0.001). This was only very slightly improved (for RA) by the addition of RA disease duration ($F_{1\text{-}293}$= 0.340, p>0.05) in the equation (from 76.9% to 77.1%), so we did not include this variable in the final model. The predictive model obtained from this analysis is:

- BF = Disease Status + Gender - 0.719 + 0.108 x Age + 1.059 x BMI
    - Where disease status: RA = 4.273; OA = 1.648; HC = 0
    - Where gender: Male = -11.294; Female = 0

Validation group

To establish external validity of our predictive model, we assessed its agreement with the measured BF in 342 patients with RA. Preliminary analyses for LIMAG revealed no heteroscedasticity, thus the LIMAG can be reported as absolute measurements (Bland and Altman, 1986). Our analyses suggested that the bias of our prediction is 0.4 (i.e., our model over-predicts BF by 0.4%) with a standard error of 3.2 ($95\%_{LIMAG}$= 6.17, coefficient of variation = 8.9; Figure 5). The difference is statistically significant (t = 2.3, p < 0.05), but the coefficient variation (CV = 8.9) is within acceptable limits.

Figure 5: Agreement between Measured and Predicted Fat in RA patients.



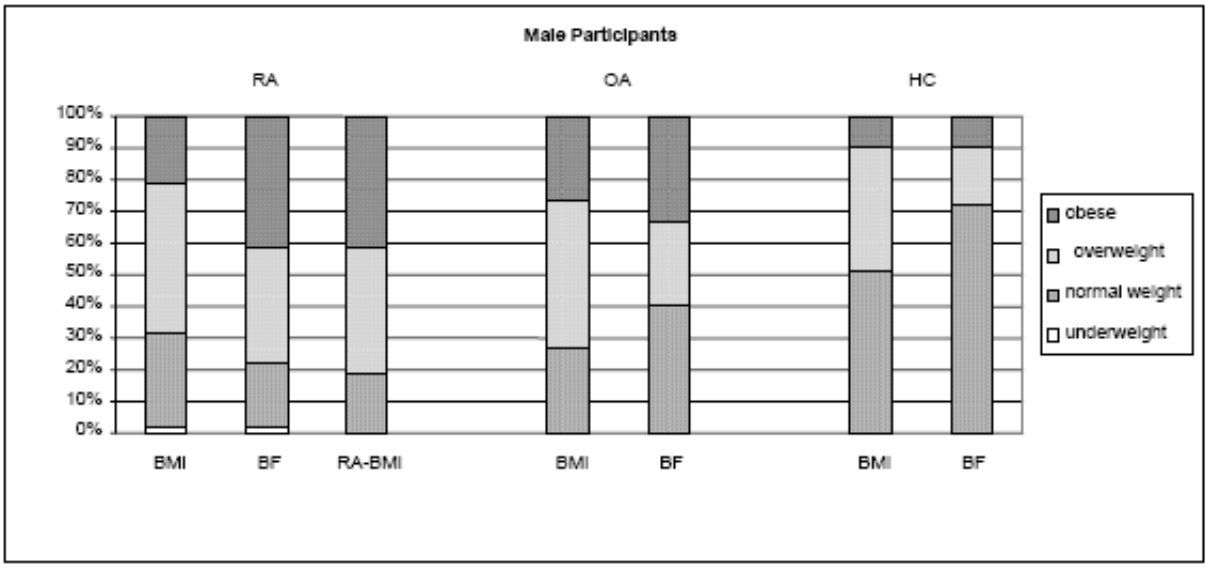
BF was measured by BIA and predicted using the formula:
BF = 4.273 + Gender - 0.719 + 0.108 x Age + 1.059 x BMI (R^2= 0.769, p<0.001).
Where gender: Male = -11.294; Female = 0

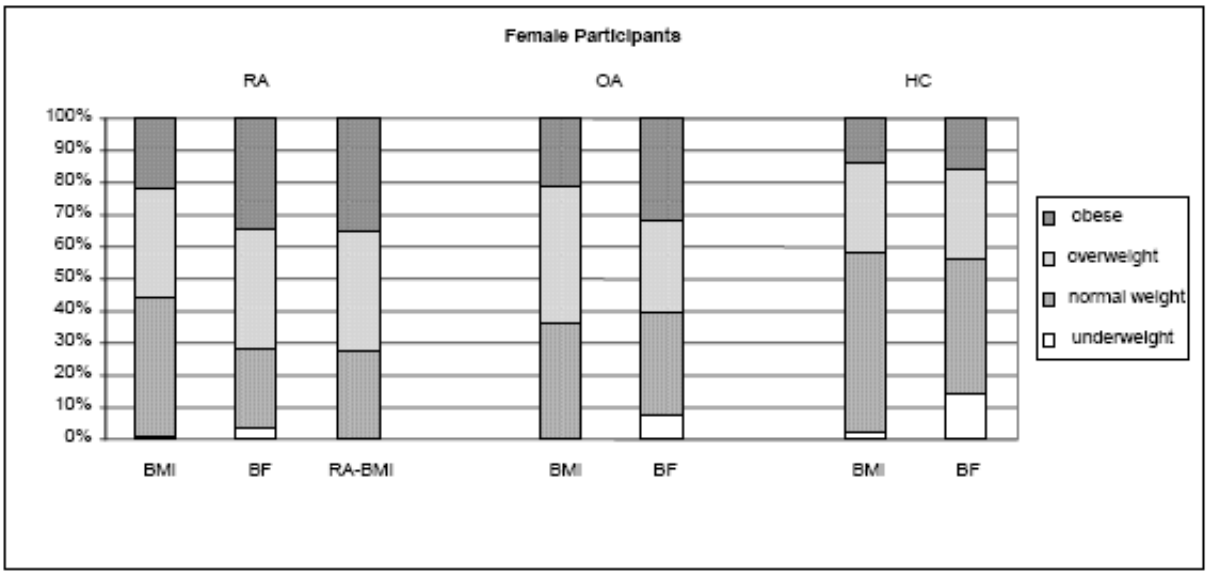
$95\%_{LIMAG}$ were 6.17 with a coefficient of variation of 8.9.

RA-specific BMI cut-off levels

The fact that patients with RA exhibited increased BF values for a given BMI compared to HC, suggested that BMI cut-off points in the RA population would be more appropriate if they were reduced by approximately 2 kg/m2 (to 23 and 28 kg/m2 for overweight and obesity respectively). We therefore compared the proportions of subjects in each group that would be correctly classified as overweight or obese using the widely accepted BMI cut-offs of 25 and 30 kg/m2 vs. the proposed (for RA) 23 and 28 kg/m2 vs. the age- and gender specific cut-off points of measured BF. This analysis showed that 9% of male and 15% of female RA patients would be misclassified as of normal weight based on traditional BMI cut-offs. Such misclassification was not a problem either for OA or HC, where if anything, BMI overestimated BF. Application of the proposed RA-specific BMI cut-offs of 23 and 28 kg/m2 corrected this misclassification (Figure 6a). A modified, RA-specific BMI chart for the classification of patients with RA into underweight, normal, overweight and obese categories was developed and is provided in Figure 6b.

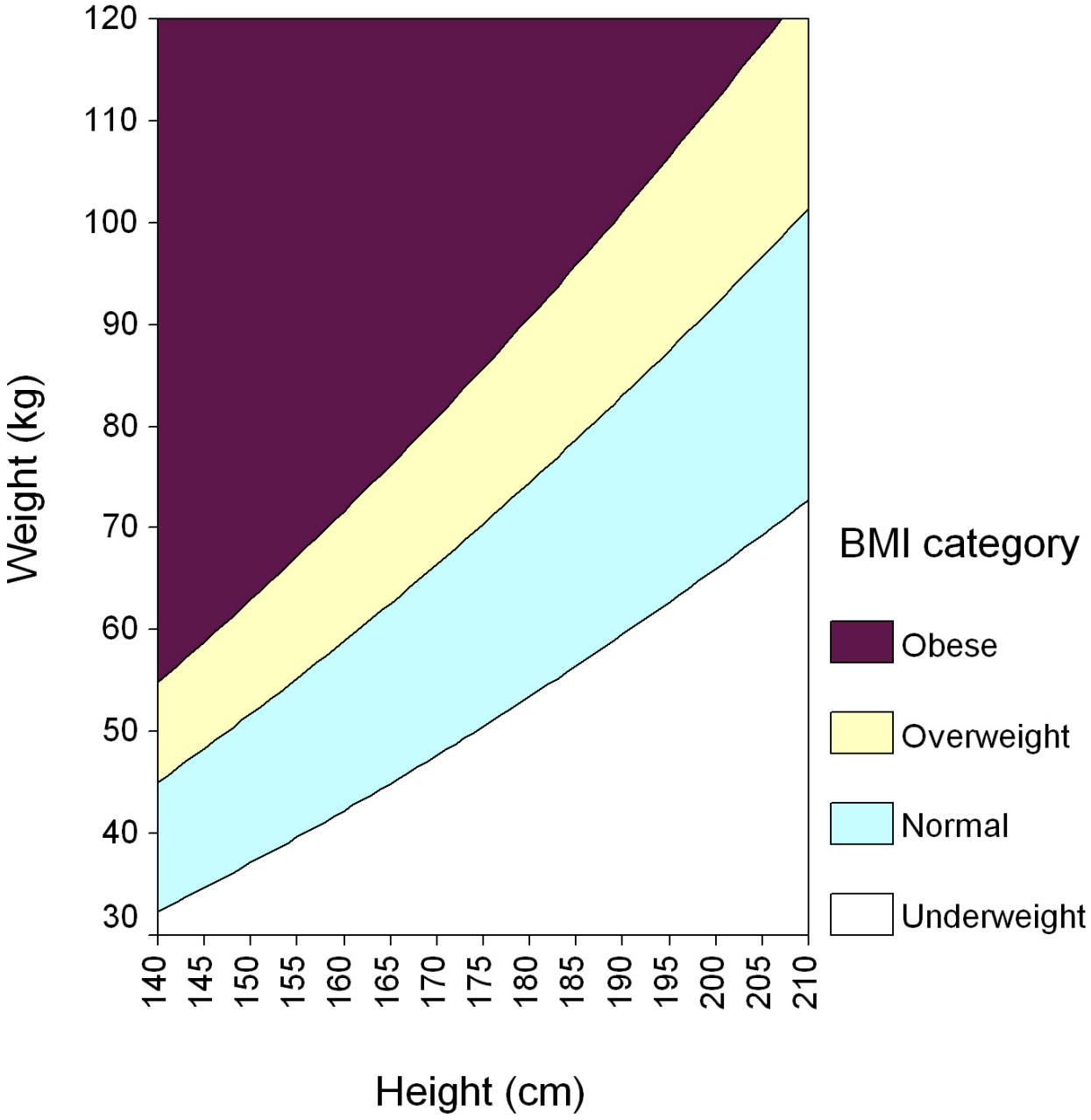
Figure 6a: Classification of male (top figure) and female (bottom figure) participants into obese, overweight, normal and underweight groups according to currently accepted BMI cut-off points (BMI), body fat content (BF) and RA-specific





BMI cut-off points (RA-BMI).
Accepting BF as the most accurate assessment of body fatness, currently accepted BMI cut-off points misclassify a significant proportion of both males and females with RA (notice the difference in the respective bars). This misclassification is corrected when the proposed RA-specific BMI cut-off points are applied.
RA: rheumatoid arthritis patients; OA: osteoarthritis patients; HC: healthy controls
BMI: classification according to existing body mass index cut-off points of 25kg/m^2 for overweight and 30kg/m^2 for obesity
BF: classification according to age and gender specific cut-off points for body fat
RA-BMI: classification according to the proposed RA-specific BMI cut-off points of 23kg/m^2 for overweight and 28kg/m^2 for obesity

Figure 6b: BMI chart developed specifically for patients with RA.



Values were calculated using the formula: BMI = weight (in kg) / height^2 (in m) for the rheumatoid arthritis-specific BMI levels identified in the present study [23kg/m^2 for overweight, 28kg/m^2 for obesity]. The generally accepted lower threshold for normal BMI [18.5 kg/m^2] was not altered.

5.1.4 Discussion

The validity of BMI as an acceptable measure of overweight or obesity, and as an accurate reflection of body fat (BF) content, has been repeatedly questioned and the need for population-specific BMI cut-off points has been highlighted (Manson *et al.*, 1995, Willett *et al.*, 1995, Blew *et al.*, 2002, Nevill *et al.*, 2004). Ideally, individualized assessment of BF should be pursued in the clinical setting, as BF percentage is a more reliable measure of fatness than BMI, at least in the general population (WHO, 2000). Indeed, our data indicate that only 58% of the variance in BMI can be predicted, as opposed to 77% in BF. BF *in vivo* can be determined via a number of methods such as underwater weighing, dual-energy x-ray absorptiometry, total body water, total body nitrogen, 40K whole body counting, and urinary creatinine excretion (Oppliger *et al.*, 1992, Demura *et al.*, 1999, Demura *et al.*, 2004). BF can also be estimated from the thickness of partial subcutaneous fat, near-infrared rays, and ultrasound (Ellis, 2000). However, none of these methods are practical for use in the routine clinical setting as they require sophisticated apparatus and specialised personnel (Demura *et al.*, 1999).

In recent years, a bioelectrical impedance method for the estimation of BF in different populations has become popular and widely recommended, as it is reliable, objective, practical, relatively inexpensive, and does not require highly trained personnel (Demura *et al.*, 1999, Demura *et al.*, 2004). The validity of this method has been confirmed in various studies (Gray *et al.*, 1989, Oppliger *et al.*, 1992, Tanaka *et al.*, 1999, Bolanowski and Nilsson, 2001, Demura *et al.*, 2004). Devices with eight tactile electrodes using single frequency electrical current, similar to the one used in this study, generate highly reproducible measurements of total BF and segmental fat distribution (Demura *et al.*, 2005). Their correlation

with the “gold standards” of dual-energy x-ray absorptiometry and hydrostatic weighing is 0.90 and 0.80 respectively, with a standard error of around 3.0, producing a co-efficient of variation of <10% (Demura *et al.*, 2004). This suggests that bioelectrical impedance measurements (especially when using eight electrodes) are valid and suitable for body composition studies (Bolanowski and Nilsson, 2001, Demura *et al.*, 2004, Demura *et al.*, 2005). Patients are usually happy to undergo such a measurement due to its simplicity and similarity to normal weighing.

In the absence of the necessary equipment or expertise, the predictive model presented here can be used to easily calculate BF of RA patients from BMI. The cross-validation of this predictive model in patients with RA is reassuring. Even though there was a statistically significant difference between the measured and the predicted BF, closer examination of the means indicates that this difference is at a level of less than 0.5% of BF with a co-efficient of variation of <10%. The statistical significance of such a small difference can be attributed to the very large number of the validation group and is clinically not important. However, the parts of the equation referring to OA patients and healthy individuals need further prospective validation in sufficiently large samples of the relevant populations.

BMI remains the most commonly used indicator of body fatness in the clinical setting, and the cut-off points of 25 and 30kg/m2 (for overweight and obesity, respectively) used for the general population are also routinely applied in RA patients. This study shows that application of these BMI cut-off points misclassified 9% of male and 15% of female RA patients in terms of actual body fatness. For a given BMI, RA patients exhibited an average 4.3% increase in BF

compared to healthy controls. In contrast, for the same level of BF, RA patients had BMI values almost 2 kg/m2 lower than those of healthy controls. We propose that BMI cut-off points in the RA population should be lowered to 23kg/m2 (from 25kg/m2) for overweight, and 28kg/m2 (from 30kg/m2) for obesity. The lowest limit for normal BMI (i.e. 18.5kg/m2) should remain unaltered, as low BMI levels have been related to increased cardiovascular risk in patients with RA (Kremers *et al.*, 2004, Escalante *et al.*, 2005). We also provide a chart for the classification of RA patients in normal, overweight and obese categories according to these BMI cut-offs, for use in the routine clinical setting (Figure 6b).

The most likely explanation for the BMI and BF differences observed in RA is rheumatoid cachexia associated with the chronic inflammatory response, given that such differences were not as prominent in OA. RA patients experience accelerated involuntary loss of fat-free mass, predominantly in the skeletal muscle, in excess of what is normally expected due to the aging process (Roubenoff *et al.*, 1994). Although the underlying mechanisms for rheumatoid cachexia remain unknown, possible contributing factors include the overproduction of inflammatory cytokines such as TNFα and IL-1 (Roubenoff *et al.*, 1994, Lecker *et al.*, 1999). Our sub-analyses within the RA population revealed that neither BMI nor BF were associated with current clinical or serological disease activity, seropositivity for rheumatoid factor (which tends to associate with more severe disease) or corticosteroid administration. This is not totally surprising as disease activity may vary within small periods of time, depending on medication and the disease itself, whereas changes in body composition are longer-term processes. On the other hand, disease duration appeared to be of some importance. It is possible that most alterations in body

composition of RA patients occur in the first few years of the disease, as it has previously been reported (Rall and Roubenoff, 2004), irrespective of disease characteristics or medical treatment.

The results of the present study are reminiscent of the observations made for Asian populations, which have significantly higher CVD risk than Caucasians: BF in Asians has been found to be 3-5% higher than that of Caucasians with similar BMI, whereas BMI was 3-4 kg/m2 lower than that of Caucasians with similar BF (Demura *et al.*, 2004). Differences in body build (trunk-to-leg-length ratio and slenderness) and in muscularity have been suggested as possible explanations for these discrepancies. As a result, new cut-off points for Asian populations have been set at 23 kg/m2 and 27 kg/m2 for overweight and obesity, respectively (WHO, 2004), and have been shown to be more sensitive in identifying Asians at increased risk for CVD (Deurenberg-Yap *et al.*, 2002).

In our participants, lowered BMI cut-off points would reflect an average reduction of 5-6 kg, or 8%, in the ideal weight (the weight one should have in order to be below the BMI cut-off for overweight). Such reductions in body weight are likely to lead to physiological benefits in the cardiovascular system: in the general population, even a 5% reduction of body weight is known to affect favourably most classical CVD risk factors (Volek *et al.*, 2002, Wilson and Grundy, 2003).

The reduced BMI cut-off points for RA suggested here may be of significance both for the management of individual patients and for further research into the cardiovascular morbidity and mortality of RA. In the clinical arena, the reduction of these thresholds would identify an additional 10-15% of people with RA as overweight or obese, and may trigger closer scrutiny for other

CVD risk factors and appropriate intervention, if necessary. Moreover, obesity, defined by the BMI, is one of the WHO criteria for the metabolic syndrome (Wilson and Grundy, 2003). Aggressive identification and reduction of classical CVD risk factors in patients with RA is an obvious strategy for reducing the increased cardiovascular mortality of this disease (Kitas and Erb, 2003). From the research perspective, the new thresholds may trigger re-analysis of previously published cohorts or further analysis of prospective cohorts as to the importance of body fat as a predictor of CVD in RA and its association with other individual risk factors.

We conclude that, in the clinical setting, body fatness of RA patients should be evaluated based on the BMI cut-off points of 23kg/m2 for overweight and 28kg/m2 for obesity. In the absence of specialised equipment, if necessary, BF of patients with RA can be estimated from BMI using the equation provided.

5.2 Associations of Obesity with Disease Activity and Severity in Patients with Established Rheumatoid Arthritis

(Published in the journal *Clinical Rheumatology* (2008) Dec 19; Epub ahead of print)

5.2.1 Introduction

RA is the commonest inflammatory arthritis (Buchan *et al.*, 1988, Feldmann *et al.*, 1996). It affects predominantly the synovial joints, which are infiltrated by chronic inflammatory cells producing cytokines such as TNFα, IL-1 and IL-6 (Feldmann *et al.*, 1996). Permanent joint damage and functional decline usually ensue (Minor and Lane, 1996), so the efforts of the scientific community have focused on symptom control and limitation of joint damage (Kitas and Erb, 2003).

An indirect effect of RA is altered body composition. Almost two thirds of RA patients suffer from a condition termed rheumatoid cachexia, which is characterised by muscle wasting in the presence of stable total body weight (Roubenoff *et al.*, 1994). As a result, RA patients present with significantly increased levels of body fat compared to healthy individuals of the same BMI (Stavropoulos-Kalinoglou *et al.*, 2007).

Adipose tissue, initially considered to be simply an energy reservoir, is now recognised as a metabolically active tissue. It secretes a number of bioactive proteins called adipokines or adipocytokines, including TNFα and IL-6 (Pi-Sunyer, 2006). This could potentially result in more active disease in obese RA patients. However, studies in patients with early RA, of up to 3 years duration, surprisingly suggest that obesity may protect against joint damage (Kaufmann *et al.*, 2003, Westhoff *et al.*, 2007, van der Helm-van Mil *et al.*, 2008). In contrast, studies in

unselected (for disease duration) RA patients suggest that obesity leads to worse quality of life (Garcia-Poma *et al.*, 2007) indicating that its potential protective effects in early RA are diminished or reversed later on in the course of the disease. The present study aimed to assess the associations of body weight and body fat with RA characteristics in patients with well-established disease.

5.2.2 Methods

Participants

Consecutive patients with RA [1987 revised ACR criteria (Arnett *et al.*, 1988)] of more than 3 years duration since symptom onset, attending routine rheumatology clinics at the Dudley Group of Hospitals NHS Trust, UK, were invited to participate. The study had Local Research Ethics Committee approval, and all volunteers provided informed consent conforming to the declaration of Helsinki. A total of 294 (male=75, female=219) volunteers were assessed. Their demographic and disease characteristics appear in Table 11.

Assessments

Standing height was measured to the nearest 0.5cm on a Seca 214 Road Rod portable stadiometer (Seca gmbh & co. kg., Hamburg, Germany). Body weight was measured to the nearest 0.1kg. Body composition was assessed by Bioelectrical Impedance, using a Tanita BC-418 MA Segmental Body Composition Analyzer, which incorporates 8 tactile electrodes (Tanita Corporation, Tokyo, Japan). BMI (kg/m2) was calculated on the basis of measured height and weight. Waist circumference was measured to the closest 0.5cm using a Seca 200 Circumference measuring tape (Seca gmbh & co. kg., Hamburg, Germany).

Table 11: Demographic and disease characteristics of all volunteers for study two [median (interquartile range) or percentage of positives]

	MALE	FEMALE
N	75	219
Age (years)	62.1 (54.2- 69.7)	62.1 (55- 68.1)
Height (cm)	173.0 (168- 178)	160 (155.5- 164)
Weight (kg)	83.6 (74.3- 93.6)	70 (60.9- 80.7)
BMI (kg/m^2)	27.6 (25.8- 30.3)	26.9 (24.1- 31.3)
BF (%)	28.8 (24.1- 31.7)	38.9 (34.5- 43.2)
ESR (mm/h)	18.5 (6.8- 31)	21 (10- 36)
CRP (mg/L)	12 (6- 22.3)	8.0 (5- 20)
DAS	4 (3.3- 4.9)	4.1 (3.3- 5.1)
HAQ	1.4 (0.5- 2)	1.6 (0.9- 2.3)
Presence of Erosions (%)	72.8	60.9
Knee Surgery (%)	6.7	8.7
Hip Surgery (%)	5.3	5.5
Wrist/Hand Surgery (%)	10.7	9.1
Elbow Surgery (%)	1.3	2.3
Shoulder Surgery (%)	2.7	1.6
Neck Surgery (%)	4	2.7
Any Surgery (%)	30.7	29.9
Disease Duration (years)	14.5 (7.8 – 20)	12 (7- 22)

N: number; BMI: body mass index; BF: body fat percentage; ESR: erythrocyte sedimentation rate; CRP: C - reactive protein; DAS: disease activity score 28; HAQ: health assessment questionnaire.

Positivity for RF and anti-CCP antibodies, as well as ESR and CRP concentrations were assessed in contemporary blood samples. Clinical disease activity and physical function were assessed by the DAS (Prevoo *et al.*, 1995) and the Anglicised version of the HAQ (Kirwan and Reeback, 1986), respectively. X-rays of hands and wrists were independently assessed by 2 rheumatologists for presence of erosions; in case of disagreement (9 in total), x-rays were jointly reviewed and a consensus opinion reached. Information on disease duration, smoking status and previous joint surgery (presence or absence) was obtained from patient interview and confirmed by reviewing the patients' hospital notes.

Data Management and Analyses

Data were inserted in a purpose-designed spreadsheet (Microsoft Excel 2003) and audited for accuracy weekly. They were exported for analysis to The Statistical Package for Social Sciences version 15.0 (SPSS Inc. Chicago, IL, USA). The Kolmogorov-Smirnov test of normality was used to assess dispersion of the variables.

Spearman's correlations were used to assess the association of weight, BMI and BF with disease activity and physical function (i.e. ESR, CRP, DAS, and HAQ). These associations were subsequently adjusted for age, gender, smoking status, RF and anti-CCP positivity and disease duration using multivariable analyses.

Thereafter, binary logistic models, with backward elimination of statistically insignificant variables, were used to test the associations of weight, BMI and BF with the presence of erosions and joint surgery. For joint surgery, independent examination of the association for each joint area (i.e. neck, shoulder, elbow, hand and wrist, hip, knee, ankle and forefoot) was pursued. The total number of joint operations was calculated and its association with BMI and BF was tested using multinomial regression. Results were standardised for age, gender, smoking status, RF and anti-CCP positivity, and disease duration.

Finally, participants were categorised according to RA-specific BMI (Stavropoulos-Kalinoglou *et al.*, 2007) into four distinct subgroups (i.e. underweight, normal-, over-weight and obese). ANOVA was used to assess differences between groups for disease activity and physical function (i.e. ESR, CRP, DAS, and HAQ). ANCOVA was used to assess the independence of these associations from age, gender, smoking status, RF and anti-CCP positivity, and

disease duration. BMI groups were also subjected to a cross-tabulation with presence of erosions and total number of operations and chi-squared analyses were performed.

Dispersion of data is reported as median (interquartile range) due to their not-normal distribution pattern. Results of the logistic models are reported as odds ratios with 95% confidence intervals (OR, 95% CI). Statistical significance was set at $p<0.05$.

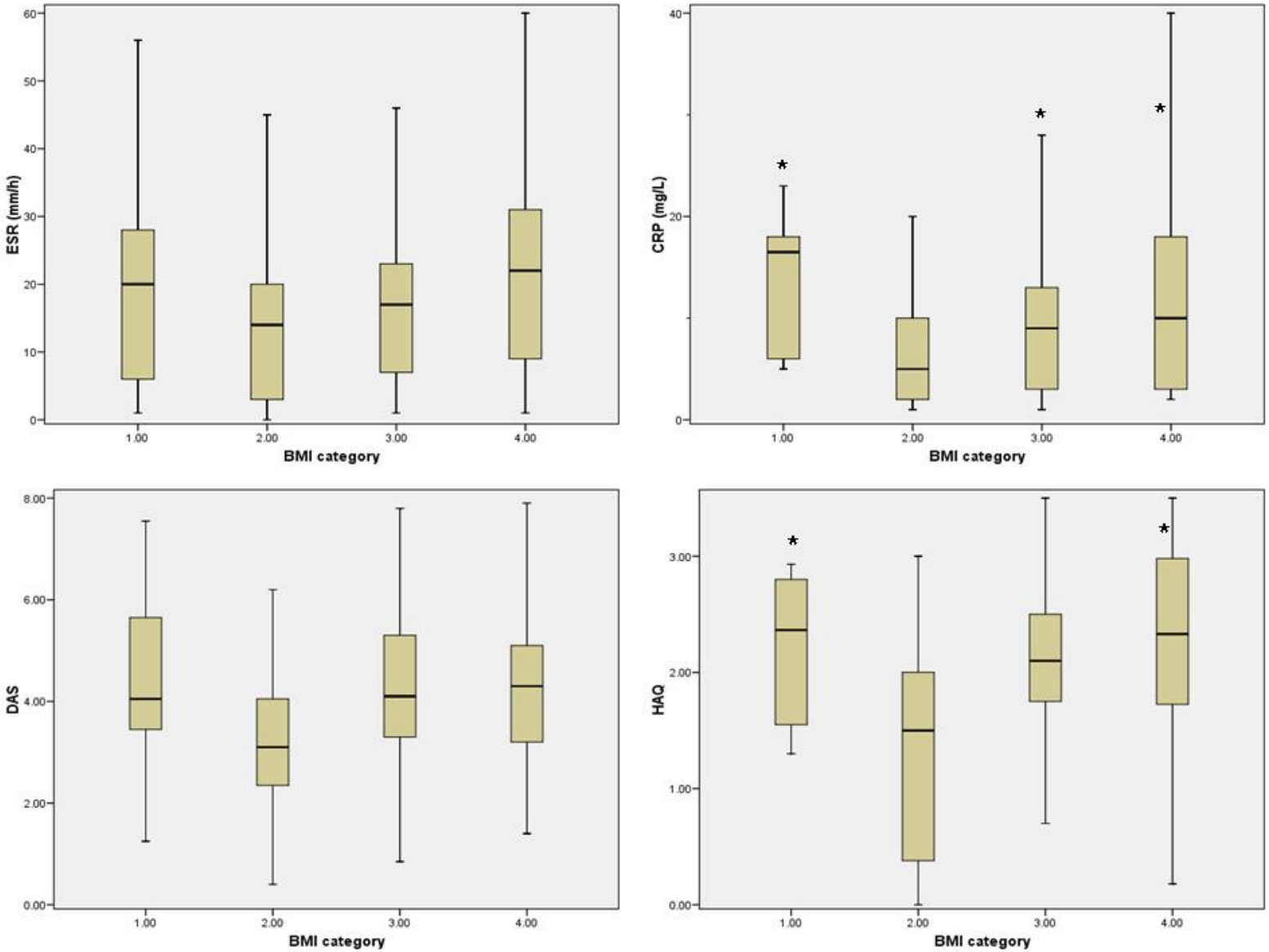
5.2.3 Results

Weight correlated significantly only with CRP ($r=0.161$, $p=0.002$). BMI correlated significantly with ESR ($r=0.145$, $p=0.012$), CRP ($r=0.178$, $p=0.002$) and HAQ ($r=0.117$, $p=0.044$). BF correlated significantly with ESR ($r=0.168$, $p=0.005$) and HAQ ($r=0.179$, $p=0.003$). After adjustment for age, gender, smoking status, RF and anti-CCP positivity, and disease duration, the association of weight with CRP was lost. BMI retained its association only with ESR ($F_{1, 290}= 7.567$; $p=0.006$) and HAQ ($F_{1, 290}= 4.059$; $p=0.045$); BF was found to associate with ESR ($F_{1, 290}= 5.767$; $p=0.017$), CRP ($F_{1, 290}= 4.162$; $p=0.042$) and HAQ ($F_{1, 290}= 7.726$; $p=0.006$). The association of BF with DAS was borderline non-significant ($F_{1, 290}= 3.888$; $p=0.055$).

Binary logistic regression showed no association of either weight, BMI or BF with presence of erosions. The same analyses revealed an inverse association of BMI with neck surgery (OR= 0.781, 95% CI: 0.637-0.958; $p=0.018$) and a positive association of BF with total knee replacement (OR= 1.146, 95% CI: 1.094-1.201; $p=0.046$), but no other associations were found. Multinomial regression models showed no association of either BMI or BF with the total number of operated joints.

Following patient grouping according to BMI into underweight, normal-weight, overweight and obese, ANOVA demonstrated significant differences in CRP ($p=0.046$) and HAQ ($p=0.034$) between the groups: patients who were either underweight or obese had significantly worse CRP and HAQ than those who had normal weight, in an almost U-shaped mode (Figure 7). A similar trend was seen with ESR and DAS also, but the differences were not significant ($p=0.095$ and $p=0.063$ respectively) (Figure 7). Chi-squared analyses failed to identify any differences between BMI subgroups for either the presence of joint erosions or total number of operations.

Figure 7: Disease activity and physical function among BMI categories



* Significant difference compared to Normal Weight ($p<0.05$)
ESR: erythrocyte sedimentation rate; CRP: C - reactive protein; DAS: disease activity score 28; HAQ: health assessment questionnaire.

5.2.4 Discussion

This study aimed to identify possible associations between weight, BMI and/or Body Fat with RA activity and severity in patients with established disease of more than 3 years duration. Weight did not associate with any of the studied variables. However, BMI significantly associated with ESR and HAQ; BF also associated with ESR, CRP and HAQ. These associations appear to be U-shaped, as both low and high BMI and BF associate with unfavourable disease activity and physical function. The differences between weight, BMI and BF in the observed associations might be explained by their varying ability to assess actual adiposity. Weight is a very generic measure that allows for large errors in the estimation of adiposity. BMI also has an inherent inability to distinguish between fat and fat free body mass (Nevill *et al.*, 2004), which makes it a less accurate marker of adiposity than BF (Wellens *et al.*, 1996), particularly in conditions such as RA, which are characterised by significant alterations of body composition (Stavropoulos-Kalinoglou *et al.*, 2007). We were unable to find any associations of either weight, BMI or BF with the presence of erosions in radiographs of the hands and wrists. BMI was inversely associated with neck surgery, while BF associated positively with total knee replacement, but their overall influence on the total number of operated joints was not significant.

Overall, the associations of adiposity with RA disease characteristics found in this study are intriguing, in that some of them are relatively easy to explain, while others seem counterintuitive. Pro-inflammatory cytokines, such as IL-1, IL-6, and TNFα, are clearly implicated in the pathogenesis and progression of RA (Buchan *et al.*, 1988, Park and Pillinger, 2007). IL-6 stimulates liver production of CRP (Castell *et al.*, 1990), a marker of inflammation and a measure of RA disease

activity, and induces further release of IL-1 and TNFα (Lyon *et al.*, 2003), which, amongst many other functions, can activate the transcription factor nuclear factor-kappa beta (NF-κβ) (Okazaki *et al.*, 2005); this is over-expressed in the inflamed synovium (Han *et al.*, 1998), and plays a central role in the initiation and progression of the chronic inflammation of RA (Tak and Firestein, 2001). The extent to which adipose tissue directly produces or indirectly induces the production of cytokines is still under intense investigation, but it is widely accepted that pro-inflammatory cytokine levels (such as IL1, IL6, and TNFα) increase and anti-inflammatory cytokine levels (such as adiponectin, IL-1 receptor antagonist, and IL-10) decrease with increasing adiposity (Juge-Aubry *et al.*, 2005). We did not directly assess cytokine levels in this study, but this mechanism would be a good explanation for the higher disease activity (in terms of ESR, CRP, or DAS) and physical dysfunction (as reflected in the HAQ) observed in participants with increased BMI or BF.

This mechanism however, does not explain the worse disease profile observed in underweight patients with very low BMI, which is in line with evidence from other studies showing increased mortality levels among underweight RA patients (Kremers *et al.*, 2004). In these patients, significantly reduced BMI is likely to be the result of highly active disease over many years (Roubenoff *et al.*, 1994) rather than vice versa, and it is interesting that low BMI appeared to associate with more neck surgery, which usually occurs in severe, uncontrolled, longstanding RA. The cross-sectional design of the present study and the relatively small number of underweight patients limit our ability to draw any definitive conclusions.

Counterintuitively, we were unable to demonstrate consistent associations between adiposity with the presence of erosions or joint surgery, despite the association of overweight and obesity with higher ESR, CRP and HAQ. Within the aforementioned limitations of the study, a possible explanation for this is the previously reported protective effect of BMI against joint damage in early RA (Kaufmann *et al.*, 2003, Westhoff *et al.*, 2007, van der Helm-van Mil *et al.*, 2008). The protective effect seems to occur mainly in RF and/or anti-CCP positive patients (Westhoff *et al.*, 2007, van der Helm-van Mil *et al.*, 2008), to be present before the diagnosis of the disease, with overweight or obese RA patients exhibiting less joint damage than their normal weight counterparts at the time of diagnosis (Westhoff *et al.*, 2007), and to continue during the first few years of the disease, with joint damage progressing less rapidly in obese than in normal-weight RA patients (Kaufmann *et al.*, 2003, Westhoff *et al.*, 2007, van der Helm-van Mil *et al.*, 2008). It is not entirely clear whether this is solely an effect of increased weight, for example through increased mechanical loading stimulating bone synthesis (Tremollieres *et al.*, 1993), or also a reflection of joint damage at the time of first diagnosis. Our data suggest that although the protective effect of increased BMI may be less pronounced in established disease, it would still appear that obese patients with established RA do not exhibit increased levels of joint destruction, despite higher levels of systemic inflammation. A partial uncoupling between the acute phase response and joint damage in RA has previously been suggested (Smolen *et al.*, 2006) and this may also be an explanation. In addition to this, it would be interesting to speculate an uncoupling between the effects of body weight (mainly reflected in weight) and adiposity (mainly reflected in BF): it is possible that the protective effects of body weight

(i.e. increased mechanical loading of the bones) continue throughout the disease, whereas the deleterious effects of adiposity (i.e. increased inflammatory load) only “kick in” later, once a critical amount of fat has accumulated through the body composition changes occurring in RA.

In conclusion and within the limitations of this cross-sectional study, in established RA, both reduced and increased adiposity seem to be related to greater disease activity and physical dysfunction but not to more joint damage. These observations are independent from several potential confounders including RF and anti-CCP positivity. Further longitudinal studies are required to address this apparent dissociation.

5.3 Associations of Obesity with Modifiable Risk Factors for the Development of Cardiovascular Disease in Patients with Rheumatoid Arthritis

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5.3.1 Introduction

Rheumatoid arthritis (RA) associates with increased risk for cardiovascular disease (CVD) (Kitas and Erb, 2003). This is most likely a combination of genetic predisposition (Gonzalez-Gay *et al.*, 2007), modifiable CVD risk factors and the inflammatory burden of the disease (Gonzalez *et al.*, 2008). RA patients have significantly higher body fat content compared to healthy individuals of the same height and weight (Stavropoulos-Kalinoglou *et al.*, 2007). This led to the development of RA-specific body mass index (BMI) thresholds for overweight and obesity that better identify RA patients with increased body fat (Stavropoulos-Kalinoglou *et al.*, 2007), and possibly CVD risk.

In the general population, obesity is a major contributor to dyslipidaemia, hypertension, and insulin resistance (Grundy *et al.*, 2004) and the underlying cause of the metabolic syndrome (MetS) (Grundy *et al.*, 2004). The associations between obesity and CVD risk factors or the MetS in RA have not been extensively investigated. Obesity in this population is usually considered a confounder, against which data should be standardised, but not as the possible underlying cause for several CVD risk factors. The aim of this study was to quantify the associations of BMI with classical CVD risk factors in a large sample of RA patients.

5.3.2 Methods

Participants

The study had ethical approval and all volunteers provided informed consent. A total of 400 (289 female) consecutive RA patients were assessed. Of them none had uncontrolled thyroid disease, but 22 were excluded due to cancer. The analyses from the remaining 378 (276 female) patients are reported: their characteristics appear in Table 12.

Table 12: Demographic and disease characteristics of all volunteers for studies three and five [median (interquartile range)]

	MALE	**FEMALE**
N	102	276
Age (years)	63.5 (13.6)	63 (14.8)
Height (cm)	173.0 (9.8)	160.0 (8.0)
Weight (kg)	83.3 (19.7)	70.0 (19.9)
BMI (kg/m^2)	27.6 (5.5)	26.7 (7.2)
DAS	4.0 (1.8)	4.2 (1.9)
HAQ	1.2 (1.8)	1.6 (1.5)
ESR (mm/h)	19.0 (30.0)	21.0 (28.0)
CRP (mg/L)	10.0 (15.0)	8.0 (13.0)
Disease Duration (years)	9.0 (15.0)	10.0 (15.0)

Assessments

Standing height, weight, BMI (kg/m2) and waist circumference were measured. CVD risk factors (BP, lipids) were assessed, smoking status noted and the Framingham 10-year CVD event probability was calculated. HOMA and QUICKI were used to determine IR. The NCEP ATP III criteria (Grundy *et al.*, 2004) were used to identify patients with the MetS. ESR, CRP, DAS, and the HAQ were also assessed.

Data Analysis

The Statistical Package for Social Sciences version 15.0 was used (SPSS Inc. Chicago, IL, USA). Dispersion of the variables was assessed using the Kolmogorov-Smirnov test. Spearman's correlations explored the associations of CVD risk factors and 10-year CVD event probability with BMI. Results were standardised for gender, age, smoking status, RA characteristics and CVD medication using univariable analyses. Multivariable analyses were used to asses the overall association of each of the possible confounders with the CVD risk factors and the 10-year CVD event probability.

Following grouping according to RA-specific BMI (Stavropoulos-Kalinoglou *et al.*, 2007) in underweight, normal-weight, overweight and obese, ANOVA was used to assess differences in BP, lipids and IR between groups. ANCOVA was then used to standardise for the same possible confounders as above. Chi-squared analyses were used to identify differences in the prevalence of each risk factor or the MetS between BMI groups. Binary logistic models were used to test the independence of these associations from the same possible confounders.

Finally, patients were grouped according to the total number of risk factors they had. ANOVA was used to assess differences in BMI among these latter groups and ANCOVA to adjust for the same possible confounders. Data are reported as median (interquartile range). Statistical significance was set to $p<0.05$.

5.3.3 Results

BMI and CVD risk factors

BMI correlated significantly with systolic BP (r=0.240, p=0.000), HDL (r=-183, p=0.000), HOMA (r=0.302, p=0.000) and QUICKI (r=-0.300, p=0.000). BMI also correlated with ESR (r=0.128, p=0.011), CRP (r=0.155, p=0.002) and HAQ (r=0.133, p=0.009), therefore results were standardised for these parameters as well as for gender, age, smoking, and CVD medication. BMI retained its association with systolic BP (F_{1-354}=23,372, p=0.000), HDL (F_{1-354}=10.439, p=0.001), HOMA (F_{1-354}=11.311, p=0.001) and QUICKI (F_{1-354}=34.678, p=0.000) and also associated with diastolic BP (F_{1-354}=7,593, p=0.006), triglycerides (F_{1-354}=4.496, p=0.035) and 10-year CVD event probability (F_{1-354}=5.857, p=0.016). Different multivariate models, using BP, lipids and IR as dependent variables, gender and smoking status as factors and BMI, age, RA characteristics and CVD medication as covariates indicated that the variance observed in all CVD risk factors was more closely associated with BMI (F_{1-354}=8.663, p=0.000), followed by lipid-lowering treatment (F_{1-354}=7.651, p=0.000), age (F_{1-354}=7.541, p=0.000), antihypertensive treatment (F_{1-354}=4.997, p=0.000) and male gender (F_{1-354}=4.707, p=0.000).

ANCOVA, with corrections for gender, age, smoking, RA characteristics and usage of CVD medication, showed significant differences between BMI sub-groups (underweight, normal-weight, overweight and obese) for systolic BP (F_{1-354}=14.707, p=0.000), diastolic BP (F_{1-354}=6.457, p=0.011), triglycerides (F_{1-354}=4.700, p=0.031), HDL (F_{1-354}=7.545, p=006), HOMA (F_{1-354}=9.720, p=0.002), QUICKI (F_{1-354}=30.332, p=0.000) and 10-year CVD event probability (F_{1-}

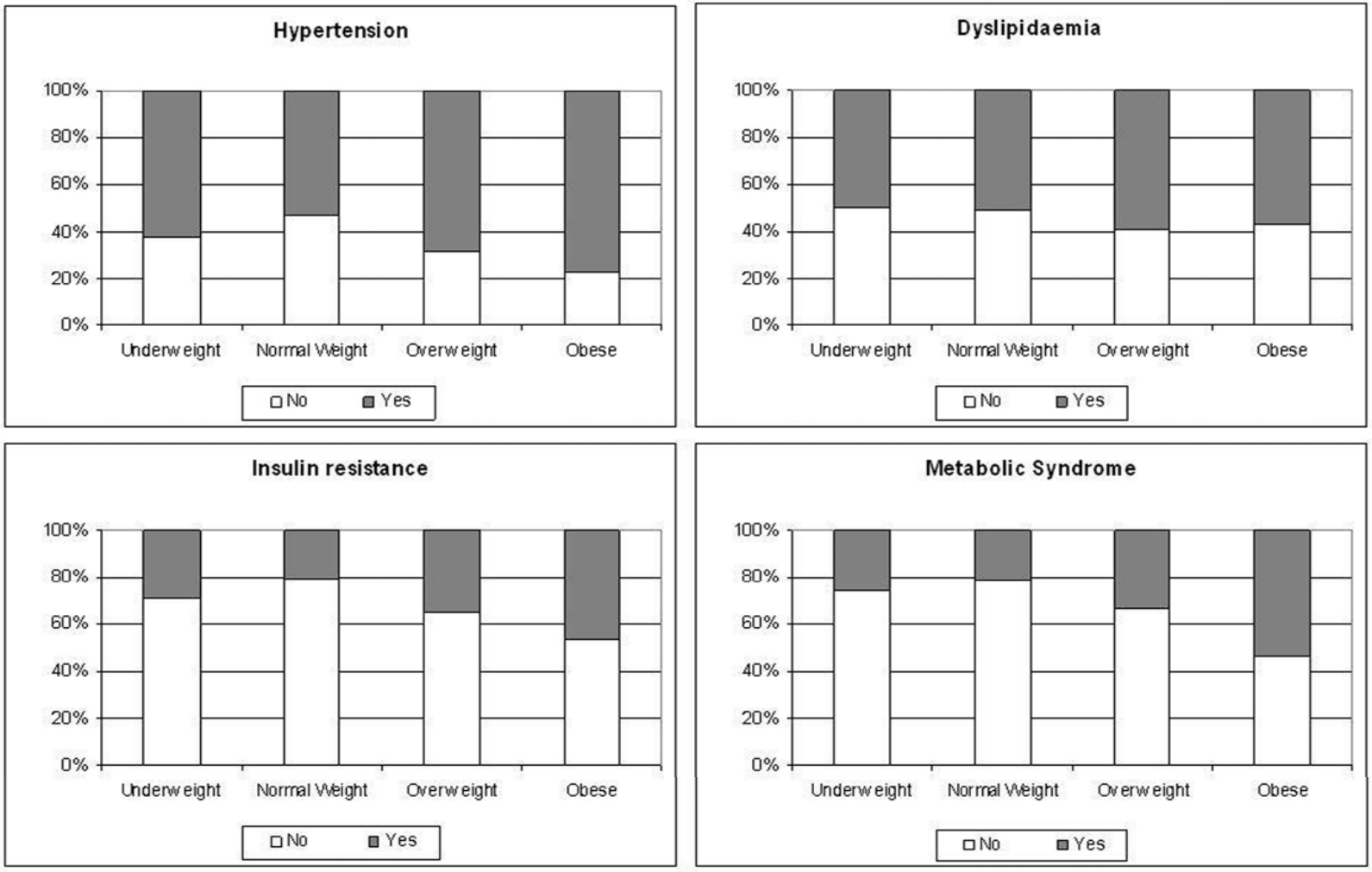
$_{354}$=3.981, p=0.046). Normal-weight patients had less CVD risk compared to other BMI groups.

BMI subgroups and the prevalence of CVD risk factors

Cross-tabulation of BMI subgroups with presence or absence of each risk factor or the MetS demonstrated significant differences between groups for the prevalence of hypertension (p=0.004), insulin resistance (p=0.005) and the MetS (p=0.000) (Figure 8). The binary logistic models indicated that BMI associated with hypertension (OR=1.28, 95% CI=1.22-1.34; p=0.001), HDL (OR=1.10, 95% CI: 1.06-1.15; p=0.025), IR (OR=1.13, 95% CI=1.08-1.18; p=0.000) and the MetS (OR=1.15, 95% CI: 1.08-1.21; p=0.000) independently of confounding factors.

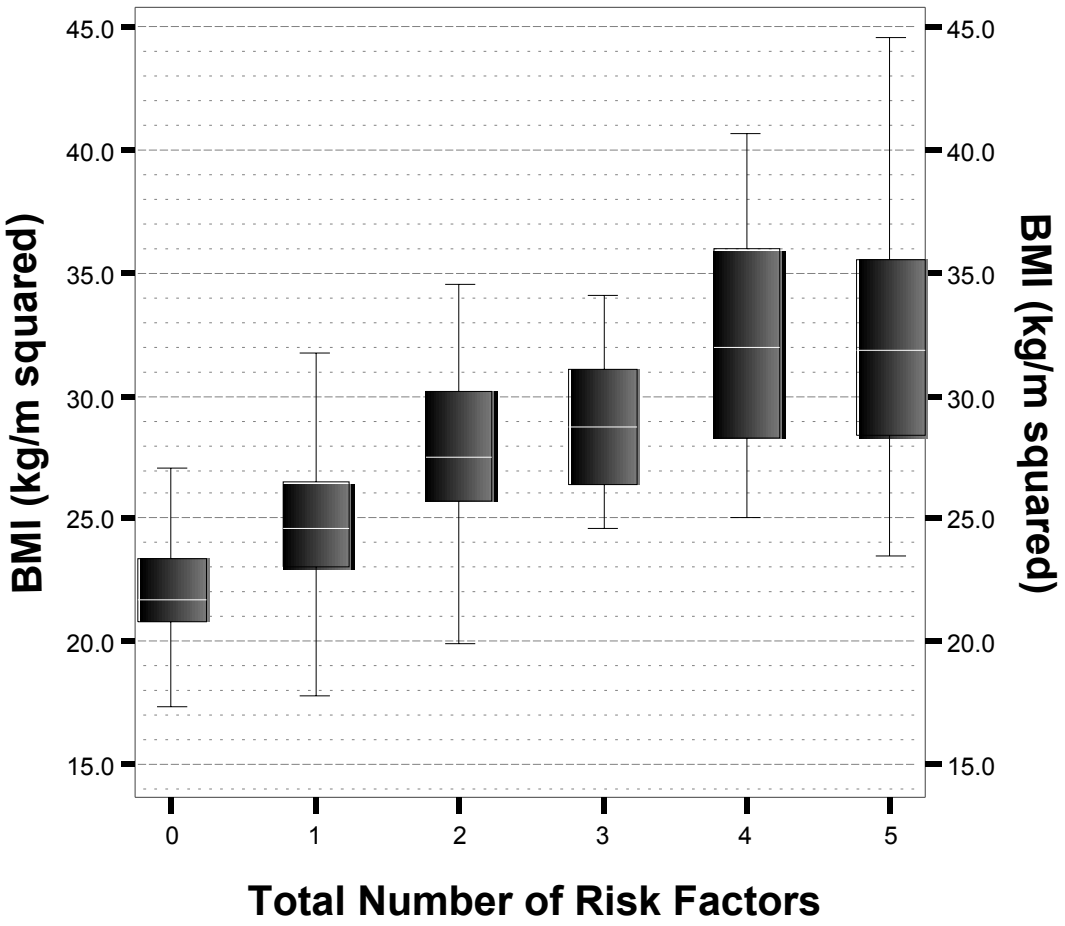
Following grouping for the total number of risk factors present, ANOVA showed significant differences in BMI between groups (p=0.000; Figure 9), while ANCOVA revealed that this association was independent of gender, age, smoking, RA characteristics and usage of CVD medication (p=0.000).

Figure 8: Prevalence of individual risk factors and the metabolic syndrome for each BMI group



Significant differences between BMI groups were found for the prevalence of hypertension (p=0.004), insulin resistance (p=0.005) and the metabolic syndrome (p=0.000). Presence of risk factors was less prevalent among normal weight patients.

Figure 9: Mean (95% confidence interval of the mean) for BMI of participants according to risk factor grouping.



Differences between groups in BMI are significant (p=0.000). Patients with lower BMI present the least number of risk factors.
Risk factors include: Hypertension, high triglycerides, low high-density-lipoprotein, insulin resistance, waist circumference

5.3.4 Discussion

These results suggest an almost linear relationship between BMI and CVD risk in this patient group, with the risk profile worsening as BMI increases, in a pattern similar to that described in the general population (Grundy *et al.*, 2004). These associations were independent of multiple confounders, and if anything, they became stronger following inclusion of CVD medication in the models.

There is no reason to suggest that the mechanisms by which obesity increases CVD risk in RA are different from those in the general population. Excess adipose tissue releases nonesterified fatty acids in the circulation, which overload the liver and muscles with lipids and increase lipolysis, while reducing glucose utilization. Circulating glucose stimulates insulin production, leading to insulin resistance (Bray *et al.*, 1998). Endothelial function is often impaired causing arterial stiffness and hypertension (Zizek *et al.*, 2001). Obesity may also increase CVD risk by reducing adiponectin, activating the rennin-angiotensin-aldosterone system, and increasing sympathetic activity and renal sodium reabsorption (Bray *et al.*, 1998). However as we did not measure any of these parameters we can only postulate about their contribution to our observations.

Recent studies in RA have shown no relation (Naranjo *et al.*, 2008) or even a “paradoxical” protective effect of obesity against CVD (Escalante *et al.*, 2005), although no potential mechanisms were described. In our study, lipid-lowering and anti-hypertensive drugs strongly associated with CVD risk factors; their inclusion in the models strengthened the association of BMI with all risk factors assessed. Such drugs, known to improve CVD risk and reduce mortality, are more frequently prescribed in obese than in non-obese individuals (Karelis *et al.*, 2004). Thus their

inclusion in the analyses of studies investigating CVD risk and outcome in RA is of paramount importance.

In the present study, neither disease characteristics nor smoking affected the associations of BMI with CVD risk. This finding is similar to our previous observations indicating that alterations in body composition of RA patients occur in the early years of the disease (Stavropoulos-Kalinoglou *et al.*, 2007) or even prior to it. This could be the case for some CVD risk factors since “the risk of coronary heart disease in RA patients precedes the ACR criteria-based diagnosis of RA” (Maradit-Kremers *et al.*, 2005). Similarly, smoking appears to confer less CVD risk in RA than in the general population (Gonzalez *et al.*, 2008). Most likely, this is a result of the smoking-induced weight-loss we recently described in RA (Stavropoulos-Kalinoglou *et al.*, 2008a) which may counteract the known negative effects of smoking on risk factors. However, disease characteristics and smoking were treated solely as possible confounders, thus their direct associations with CVD risk in RA cannot be assessed in this study.

An important finding of the present study is the BMI level at which CVD risk increases. Patients with one risk factor had a median BMI of <25kg/m2 whereas those with the MetS <30 kg/m2 and by applying general BMI thresholds would be classified as normal-weight and overweight respectively; however, based on RA-specific BMI thresholds they would be classified as over-weight or obese. This could be important in routine clinical practice, where such classifications may be used to target patients at increased risk for screening, early identification and management of risk factors.

In the general population, weight-loss can reverse the adverse effects of obesity. However, in a population with significant muscle wasting, such as RA, the

type of weight-loss intervention has to be carefully considered. Among existing weight-loss regimens, exercise and especially resistance training, is the only one proven to increase muscle mass in the general population (Franz *et al.*, 2007) and may be applied in RA patients without aggravating their disease (Metsios *et al.*, 2008c). Moreover, exercise is known to further reduce CVD risk irrespective of weight-loss (Gaesser, 2007). Research focusing on weight-loss interventions and their effects on CVD in RA are necessary.

Within its limitations, this study shows that increasing BMI in RA patients associates with increased CVD risk. The use of RA-specific BMI thresholds better identifies RA patients at increased CVD risk. Weight-loss regimens specific for RA patients need to be developed and evaluated.

5.4 Subtypes of Obesity in Rheumatoid Arthritis

5.4.1 Introduction

Obesity is a condition that develops from a quantitative imbalance between energy intake and energy expenditure and is characterised by increased adiposity (Abdel-Hamid, 2003); it associates with metabolic abnormalities such as insulin resistance and obese individuals are at a higher risk for CVD (Bray, 1995). However, not all obese individuals exhibit such metabolic abnormalities or increased CVD risk and not all normal-weight individuals are metabolically healthy or free from CVD (Karelis *et al.*, 2004).

Two distinct subtypes of obesity have been identified (Karelis *et al.*, 2004). The first subtype, termed metabolically-obese but normal-weight (MONW), consists of individuals with normal weight who exhibit increased levels of insulin resistance and accumulation of other modifiable CVD risk factors (i.e. hypertension, dyslipidaemia) (Ruderman *et al.*, 1981, Ruderman *et al.*, 1982). The second subgroup, termed metabolically-healthy but obese (MHO), exhibits increased levels of BMI and BF but no other metabolic complications (Sims, 2001). The prevalence of MONW in the general population ranges between 5% (Park *et al.*, 2003) and 45% (Molero-Conejo *et al.*, 2003), however most studies suggest that ~15% of normal weight individuals are MONW (Conus *et al.*, 2007). Similarly, among obese individuals, ~20% are MHO (Ferrannini *et al.*, 1997, Bonora *et al.*, 1998, Karelis *et al.*, 2004). Significantly, both subtypes associate with different inflammatory profile. MONW exhibit increased levels of inflammation compared to other normal-weight individuals (Hyun *et al.*, 2008), while MHO

exhibit reduced levels of inflammation compared to other obese individuals (Shin *et al.*, 2006, Karelis and Rabasa-Lhoret, 2008).

RA is the commonest inflammatory arthritis and associates with increased joint pain and stiffness, leading to irreversible joint damage and functional disability (Alamanos and Drosos, 2005). The inflammation of the disease, coupled with the classical CVD risk factors (Gonzalez *et al.*, 2008), has been implicated in atherosclerotic and thrombogenic processes in RA patients (Sattar *et al.*, 2003). Obesity, a common cause of increased CVD risk, is frequently observed in RA (Stavropoulos-Kalinoglou *et al.*, 2007); increased muscle breakdown caused by inflammation (Metsios *et al.*, 2008b) leads to significant increases in body fat of RA patients (Stavropoulos-Kalinoglou *et al.*, 2007). Insulin resistance is also frequently reported in this population (Sattar *et al.*, 2003) as are the other modifiable CVD risk factors (Panoulas *et al.*, 2007).

In RA, obesity associates with increased prevalence of CVD risk factors (Stavropoulos-Kalinoglou *et al.*, 2008b), increased disease activity and diminished quality of life (Garcia-Poma *et al.*, 2007); however, some studies report a protective effect of it against CVD death (Escalante *et al.*, 2005) and joint damage. The existence of subtypes of obesity in this population could possibly explain this discrepancy and identification of such individuals could affect the clinical intervention they receive. Furthermore, effective control of inflammation could improve the metabolic status of RA patients and consequently affect their obesity subtype. Thus the aim of this study is 1) to estimate the prevalence and identify predictors of the subtypes of obesity in RA, 2) to investigate their associations with body composition, CVD risk factors, and RA disease

characteristics and 3) to assess the effects of anti-tumour necrosis factor alpha (anti-TNFα) treatment on the metabolic status of MONW and MHO.

5.4.2 Methods

Participants

Consecutive patients attending routine rheumatology clinics at the Dudley Group of Hospitals NHS Trust, UK, were invited to participate. The study had Local Research Ethics Committee approval by the Dudley Ethics Committee, and all volunteers provided informed consent. A total of 400 (male=111, female= 289) volunteers with RA [1987 revised ACR criteria (Arnett *et al.*, 1988)] were assessed. Of them 22 (male=9, female=13) were excluded from analysis as they had cancer. There were no patients with uncontrolled thyroid disease in this cohort. The analyses from the remaining 378 (male= 102, female=276) volunteers are reported. Their demographic and disease characteristics appear in Table 12.

Further to the data collected during their initial assessments, clinical notes of patients characterised as MONW or MHO who embarked on anti-TNFα treatment within the duration of this study were retrospectively examined. Data on BMI, CVD risk factors and RA disease characteristics were collected for these patients following 6-8 months of treatment. The total of six (three MONW and three MHO) patients fulfilled these criteria. Their results were compared against those of age, gender, BMI, disease duration and smoking status matched (at initial assessment) patients that were not categorised as either MONW or MHO but also embarked on anti-TNFα treatment.

Assessments

Standing height was measured to the nearest 0.5cm on a Seca 214 Road Rod portable stadiometer (Seca gmbh & co. kg., Hamburg, Germany). Body weight was measured to the nearest 0.1kg. Body composition was assessed by Bioelectrical Impedance, using a Tanita BC-418 MA Segmental Body Composition Analyzer, which incorporates 8 tactile electrodes (Tanita Corporation, Tokyo, Japan). BMI (kg/m2) was calculated on the basis of measured height and weight. The recently published RA-specific BMI cut-off points (i.e. 23kg/m2 for overweight and 28kg/m2 for obesity) were used to classify them as under-, normal-, over-weight, or obese (Stavropoulos-Kalinoglou *et al.*, 2007). Waist circumference was measured using a Seca 200 Circumference measuring tape (Seca gmbh & co. kg., Hamburg, Germany). Cut-offs for increased waist circumference were >102cm for males or >88cm for females (Lean *et al.*, 1995).

BP, blood lipids, glucose and insulin were also assessed. Patients with systolic BP≥140 and/or diastolic BP≥90 and/or receiving antihypertensive medication, were characterised as hypertensive (Williams *et al.*, 2004). Patients with increased levels of triglycerides (>1.7mmol/L), total cholesterol (>6.2mmol/L), low-density-lipoprotein (LDL >4.13mmol/L), decreased levels of high-density-lipoprotein (HDL <1.03mmol/L) or receiving lipid lowering therapy were characterized as dyslipidaemic (Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults, 2001). Patients with HOMA ≥ 2.5, QUICKI ≤ 0.333, presence of diabetes mellitus, or use of anti-diabetic medication were considered as insulin resistant. Patients exhibiting normal BMI (i.e. 18.5-23kg/m2) but increased levels of IR where characterised as MONW. Obese patients (BMI>28kg/m2) with normal insulin sensitivity where characterised as

MHO. Smoking status was also noted and the Framingham 10-year CVD event probability score was calculated as previously described (Anderson *et al.*, 1991).

Contemporary serological inflammation was assessed by ESR and CRP. DAS was used to assess clinical disease activity (Prevoo *et al.*, 1995). The anglicised version of the HAQ (Kirwan and Reeback, 1986) was used to measure functional disability as a proxy for disease severity. Disease duration was recorded from review of the clinical notes.

Data Management and Analyses

Data were inserted in a purpose-designed spreadsheet (Microsoft Excel 2003) and audited for accuracy weekly. They were exported for analysis to The Statistical Package for Social Sciences version 15.0 (SPSS Inc. Chicago, IL, USA). The Kolmogorov-Smirnov test of normality was used to assess dispersion of the variables. Dispersion of data is reported as median (interquartile range) due to their abnormal distribution pattern. Results of the logistic models are reported as odds ratios with 95% confidence intervals (OR, 95% CI). Statistical significance was set at $p<0.05$.

Prevalence and predictors of subtypes of obesity in RA

The frequency of occurrence of each subgroup was examined in the descriptive statistics of our sample. Regression analyses were used to identify significant predictors for subtype allocation. Specifically: age, gender, BF, FFM, waist circumference, ESR, CRP, DAS, HAQ, disease duration, smoking habits and medication were included in several different logistic models. Insignificant variables were eliminated until only the significant predictors remained in the model.

Differences in body composition, CVD risk and RA disease characteristics between subtypes of obesity

ANOVA was used to test for differences between MONW and normal weight-metabolically healthy participants as well as between MHO and metabolically unhealthy obese patients in: body composition, conventional CVD risk factors, 10-year CVD event probability, and RA disease characteristics. The results were corrected for age, gender, BMI, disease duration, smoking status and medication.

Effects of anti-TNFα medication on subtypes of obesity

Initially, repeated measures ANOVA was used to assess the effects of anti-TNFα treatment on BMI and insulin resistance of MONW, MHO and their age, gender, BMI, disease duration and smoking status matched counterparts. The same analyses were then used to assess the effects of anti-TNFα on blood pressure, lipids and RA characteristics among the same participants.

5.4.3 Results

Prevalence and predictors of subtypes of obesity in RA

Among our normal-weight participants 21 (22.4%) exhibited insulin resistance and were characterised as MONW. Normal-weight patients with higher BF were more likely to be characterised as MONW (OR= 2.781, 95% CI: 2.073- 4.009; p=0.007) as were patients with larger waist circumference (OR= 1.482, 95% CI: 1.029-2.134; p=0.035). On the contrary, patients with increasing levels of FFM (OR= 0.221, 95% CI: 0.060-0.815; p=0.023) and those receiving anti-TNFα treatment (OR= 0.479, 95% CI: 0.200- 0.855; p=0.012) were less likely to be MONW.

Among the obese participants 24 (19.9%) had normal insulin resistance and where classified as MHO. Low BF (OR= 1.603, 95% CI: 1.186-2.165; p=0.002), small waist circumference (OR= 1.769, 95% CI: 1.251-2.506; p=0.001)

and increased FFM (OR= 1.173, 95% CI: 1.039-1.498, p=0.013) were significant predictors of MHO allocation. Also, current smokers were more likely to be MHO compared to never smokers (OR= 2.778, 95% CI: 1.937-3.32; p=0.014) or ex-smokers (OR=1.667, 95% CI: 1.141-2.503; p=0.040).

Differences in body composition, CVD risk and RA disease characteristics between subtypes of obesity

Compared to their metabolically healthy normal-weight counterparts, MONW individuals had significantly higher BF (p=0.010) and larger waist circumference (p=0.003). They also exhibited higher systolic blood pressure (p=0.030), triglycerides (p=0.004), glucose (p=0.000) and insulin (p=0.000). Total cholesterol did not differ significantly between groups but MONW had significantly lower HDL cholesterol (p=0.012). Their overall 10-year CVD event probability was also significantly higher compared to that of other normal-weight participants (p=0.016). Finally, MONW individuals scored significantly higher in HAQ (p=0.002) and presented higher levels of ESR (p=0.043). A trend for higher DAS and CRP among MONW compared to metabolically healthy normal-weight patients was also observed but it was borderline insignificant (p=0.073 and p=0.062 respectively). Corrections for age, gender, BMI, disease duration and smoking status did not alter the results. However, following correction for gender, FFM was found to differ significantly between these two subtypes of obesity ($F_{1, 96}$ = 4.037; p=0.038).

On the other hand, MHO individuals had significantly lower BF (p=0.008) and smaller waist circumference (p=0.022) compared to other obese participants. They also differed in systolic BP (p=0.034), triglycerides (p=0.000), glucose (p=0.000), insulin (p=0.000) and 10-year CVD event probability (p=0.000).

Moreover, MHO had significantly lower HAQ scores (p=0.002) and ESR levels (p=0.045) than the other obese participants. They also tended to have lower DAS scores (p=0.070) and CRP levels but the differences were not significant. Again following correction for gender, FFM was found to differ significantly between MHO and obese ($F_{1,\ 110}$= 8.344; p=0.005) Correction for age, gender, BMI, disease duration and smoking status did not influence the results in any other way. ANOVA results for all subtypes are presented analytically in Table 13.

Effects of anti-TNFα medication on subtypes of obesity

Anti-TNFα treatment did not affect BMI of any of the participants irrespective of subtype allocation (p>0.05). Repeated measures ANOVA indicated that inflammation (as indicated by ESR, CRP and DAS) was equally reduced in all groups (p for differences between groups >0.05 in all cases). However, it resulted in greater decreases in HOMA (p=0.031) and QUICKI (p=0.025) in MONW patients compared to other normal weight patients. Similarly, anti-TNFα treatment resulted in greater decreases in systolic BP (p=0.048) and triglycerides (p=0.034) in these patients. On the other hand, it marginally improved insulin sensitivity in both MHO and obese individuals; however no differences in the magnitude of improvements between the two groups were observed (p>0.05 for both HOMA and QUICKI). Similarly no differences between groups in the changes in other CVD risk factors were observed.

Table 13: ANOVA results for the differences in anthropometric, CVD and RA characteristics between subtypes of obesity (median [interquartile range])

	Normal weight	MONW	MHO	Obese
N	21	73	24	97
Body Fat (%)	30	33.8*	41.2#	43.4
	(24.4-34.2)	(28.6-36.5)	(31.7-43.4)	(35.3-47.05)
Fat Free Mass (kg)	39.3	38.6^	50.5⌂	50
	(36.7-43.6)	(35.8-47)	(47.8-58.7)	(44.9-63.1)
Waist Circumference (cm)	83	86.5*	104#	110
	(80-86)	(83.5-92.8)	(101-111)	(105-115.5)
Systolic BP (mmHg)	131	141.5*	140#	148
	(122-144)	(135-150)	(125-153)	(137.5-167)
Diastolic BP (mmHg)	79	75.5	80	84
	(70-83)	(70-84.3)	(71-86)	(73-90)
Triglycerides (mmol/L)	1	1.4*	1.1#	1.5
	(0.8-1.3)	(1.1-1.8)	(0.9-1.6)	(1-2.3)
Total Cholesterol (mmol/L)	5.2	5.4	5.4	5.8
	(4.7-5.5)	(5-6.3)	(4.2-5.6)	(4.4-6.9)
HDL Cholesterol (mmol/L)	1.7	1.5*	1.6	1.4
	(1.5-1.9)	(1.4-1.7)	(1.3-1.8)	(1.2-1.7)
Glucose (mmol/L)	4.8	5.3*	4.9#	5.5
	(4.5-5)	(4.7-5.4)	(4.6-5.1)	(4.9-6.2)
Insulin (pmol/L)	35	60.8*	50#	127
	(23.5-49)	(36.3-88)	(35-57.1)	(91.5-180.5)
CVD (%)	3	6*	5#	11
	(1-5.5)	(3.3-12.8)	(2-8)	(5-19)
HAQ	1.1	1.5*	1.6#	1.9
	(0.3-1.6)	(1.1-2.2)	(0.7-2.1)	(1.3-2.3)
DAS	3.6	4	4.2	4.6
	(2.9-6.1)	(3.1-4.7)	(3-5)	(3.4-5.5)
ESR (mm/h)	16	20*	20#	25
	(8-28)	(12.5-30.5)	(9-31)	(15.5-36)
CRP (mg/L)	7	12.5	13	15
	(4-15.5)	(4-16.8)	(5-19)	(6-24)
Disease Duration (years)	9	11.5	8	10
	(3-17.5)	(4.3-25.8)	(3-15)	(5-15.5)

* Significantly different to normal weight ($p<0.05$)
^ Significantly different to normal weight following correction for gender ($p<0.05$)
# Significantly different to obese ($p<0.05$)
⌂ Significantly different to obese following correction for gender ($p<0.0$
MONW: metabolically obese normal weight; MHO: metabolically healthy obese; BP: blood pressure; HDL: high density lipoprotein; CVD: 10-year cardiovascular event probability; HAQ: health assessment questionnaire; DAS: disease activity score 28; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein

5.4.4 Discussion

This study is the first to investigate the existence of subtypes of obesity in RA. Its aims were 1) to estimate the prevalence and identify predictors of the subtypes of obesity in RA, 2) to investigate their associations with body composition, CVD risk factors, and RA disease characteristics and 3) to assess the effects of anti-tumour necrosis factor alpha (anti-TNFα) treatment on the metabolic status of MONW and MHO. Our results suggested a prevalence of 22.4% for MONW and 19.9% for MHO. BF, waist circumference and FFM were significant predictors for subgroup allocation for both MONW and MHO. Interestingly though, anti-TNFα medication was a predictor only for MONW and smoking only for MHO. MONW had significantly increased CVD risk and worse RA characteristics compared to other normal weight participants. On the contrary, MHO had significantly decreased CVD risk and better RA characteristics compared to other obese patients. Finally, anti-TNFα medication improved metabolic status and CVD risk of MONW but not that of MHO or obese participants.

In the general population, the prevalence of MONW is less than that in our patients (15% vs. 22.4%); however that of MHO is roughly the same (i.e. ~20%). The underlying mechanism for the existence of subtypes of obesity is not clear. The prevailing theory suggested increased overall as well as abdominal/visceral fat deposition in MONW and decreased in MHO compared to other normal-weight and obese individuals respectively (Karelis *et al.*, 2004). The different inflammatory profiles observed among different subtypes are thought to be the effect rather than the cause of altered fat deposition (Conus *et al.*, 2007).

From a clinical point of view, the reason for studying the subtypes of obesity lies in the clinical interventions these individuals receive. CVD risk of

MONW usually remains undiagnosed, due to their “healthy” stature, and thus they do not receive the appropriate treatment (Conus *et al.*, 2007). On the contrary, MHO might receive treatment that they do not need and could possibly trigger some different health conditions (Karelis *et al.*, 2004). Identification of predictors of subtype allocation is thus very important. In our participants, BF and waist circumference (an indicator of abdominal/visceral fat) were significant predictors for subtype allocation indicating that the cause of this phenomenon is similar in RA patients and the general population. FFM was also a significant predictor for subgroup allocation among RA patients. From the existing literature it is not clear whether reduced FFM is directly implicated in insulin resistance; it is however well established that the main cause for FFM reduction in the general population is physical inactivity (Bray and Bellanger, 2006). Such inactivity can result in insulin resistance directly, via reduced insulin action on the muscle, or indirectly due to increases in BF (Bray and Bellanger, 2006). In the RA population, inflammation associated with the disease can also lead to reduced FFM (Roubenoff *et al.*, 1994). Chronic overproduction of cytokines, especially of TNFa, is implicated in rheumatoid cachexia; a condition characterised by involuntary loss of FFM at the presence of stable or slightly increased body weight (Roubenoff *et al.*, 1994). This eventually leads to increased overall BF (Stavropoulos-Kalinoglou *et al.*, 2007). Moreover acute increases in pro-inflammatory cytokines during active disease can directly increase insulin resistance in RA patient (Dessein *et al.*, 2002, Sattar *et al.*, 2003). This suggests a more significant role of inflammation in insulin resistance of RA patients compared to the general population and it could be the explanation for the slightly increased prevalence of MONW among RA patients. To further support that, anti-TNFa medication -which is known to control

inflammation- was found to associate only with the normal-weight/MONW groups. In our cross-sectional observations anti-TNFa reduced the chances of a normal-weight patient to be insulin resistant. In the retrospective branch of the study, anti-TNFa was found to improve insulin resistance and overall CVD risk of MONW more than that of other normal weight participants. Interestingly though, anti-TNFa medication or any other marker of disease activity, did not affect subtype allocation in obese participants. Additionally, following 6-8 months of anti-TNFa treatment, metabolic and CVD parameters of MHO and obese individuals were altered in similar fashion leading to the notion that among obese individuals, adiposity rather than inflammation is the major cause of insulin resistance. The fact that obese smokers had less chances of being insulin resistant compare to never- or ex-smokers further supports that. As we have recently shown (Stavropoulos-Kalinoglou *et al.*, 2008a), active smoking relates to reduced BMI and BF in RA patients whereas smoking cessation relates to the opposite.

From a scientific point of view, the importance of studying the subtypes of obesity lies on the mechanisms that lead to their altered CVD and inflammatory profiles. As stated previously, MONW individuals present with increased levels of visceral fat compared to other normal weight individuals (Conus *et al.*, 2007) whereas MHO have significantly less visceral fat compared to their obese counterparts (Sims, 2001). The reason for this inter-individual differentiation is not known, however genetic factors and age of obesity onset have both been implicated (Ailhaud and Hauner, 1998, Freedland, 2004). Visceral fat is mainly composed of white adipose tissue (WAT) (Matsuzawa, 2005). In contrast to brown adipose tissue, which is mainly developed during the embryonic and early infant years, WAT is developed throughout the life of an individual and is the major site

for excessive fat storage (Ailhaud and Hauner, 1998). In order to facilitate fat storage, adipocytes of WAT have to expand in size, as differentiation of pre-adipocytes to adipocytes in this tissue is limited (Freedland, 2004). While smaller adipose cells are insulin sensitive, when they are enlarged they develop insulin resistance (Freedland, 2004). Insulin resistance in such cells is though to be a defence mechanism developed to protect them against excessive fat storage (Freedland, 2004). However, this mechanism deviates fat storage to non-fat tissues, such as liver, muscles and the heart (i.e. ectopic fat storage), and is also reflected in the circulation as hyperlipidaemia (Freedland, 2004). Furthermore, adipose tissue with enlarged adipocytes shows consistent low-grade inflammation (Matsuzawa, 2005). Recent studies have attributed this to localised tissue hypoxia (Trayhurn and Wood, 2004, Hosogai *et al.*, 2007, Ye *et al.*, 2007). WAT has limited vascularisation and a small number of mitochondria (Ailhaud and Hauner, 1998) resulting in its relatively poor oxygenisation; enlargement of adipose cells further limits oxygen delivery to them (Trayhurn and Wood, 2004). This results in a dysregulation of the expression and secretion of pro-and anti- inflammatory cytokines (i.e. increased IL-6 and TNFα and decreased adiponectin) (Hosogai *et al.*, 2007, Wang *et al.*, 2007, Ye *et al.*, 2007, Trayhurn *et al.*, 2008). Increased levels of pro-inflammatory molecules have been implicated in the process of atherosclerosis (Ross, 1999), specifically by inducing endothelial dysfunction (Alexander, 1995, Bhagat and Vallance, 1997), the first step of atherosclerosis. Endothelial dysfunction is also known to associate with hypertension (Rizzoni, 2002). Even though in this study we did not assess adipose hypoxia or any adipokines, the above described mechanism, responsible for the differing CVD

and inflammatory profiles between subtypes of obesity, should also be valid in the RA population.

In conclusion and within the limitations of this study our results suggest that the prevalence of MONW in RA is slightly increased compared to that in the general population, while that of MHO is similar. Total and central adiposity are significant predictors for both. Inflammation seems to associate more with insulin resistance in MONW than in obese. Adiposity seems to be the major cause of insulin resistance in the latter subtype. The underlying mechanisms for the existence of subtypes of obesity in RA are far from clear. Further research focusing on specific adipose depots and their metabolic properties should be conducted.

5.5 Association of Cigarette Smoking with Body Weight and Muscle Mass of Patients with Rheumatoid Arthritis

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5.5.1 Introduction

RA, the commonest inflammatory arthritis, associates with altered metabolism (Roubenoff *et al.*, 1994). Compared to healthy controls, RA patients exhibit elevated resting energy expenditure (REE) and enhanced muscle catabolism (Rall and Roubenoff, 2004). Such changes may lead to rheumatoid cachexia, i.e. involuntary loss of FFM with proportional increase of BF, in the presence of stable body weight (Walsmith and Roubenoff, 2002, Metsios *et al.*, 2006). Body composition changes, particularly BF increase, may remain largely undetected by traditional assessments, such as the BMI (Stavropoulos-Kalinoglou *et al.*, 2007). Increased BF, together with reduced levels of physical activity due to joint inflammation and damage (Walsmith and Roubenoff, 2002, Metsios *et al.*, 2008c), are associated with several co-morbidities, including CVD (Orzano and Scott, 2004, Poirier *et al.*, 2006) as well as increased mortality (Walsmith and Roubenoff, 2002).

Cigarette smoking is an important risk factor for several diseases (Frieden and Bloomberg, 2007). It is also known to decrease body weight in healthy individuals by reducing appetite, and increasing REE (Akbartabartoori *et al.*, 2004). In contrast, smoking cessation may associate with significant weight increase, which constitutes a major deterrent to smoking control (Eisenberg and Quinn, 2006).

We have recently demonstrated that smoking further increases REE in RA (Metsios *et al.*, 2008a), and this could potentially augment rheumatoid cachexia in these patients. Given the RA-related alterations in body composition and the comorbidity associated with them, examination of potential contributors to muscle wasting, such as smoking, is important. The aim of this cross-sectional study was to detect potential associations between smoking and body weight, body composition and rheumatoid cachexia in RA patients.

5.5.2 Methods

Participants

Consecutive patients attending routine rheumatology clinics at the Dudley Group of Hospitals NHS Trust, UK, were invited to participate. All applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. The study had Local Research Ethics Committee and Research and Development Directorate approvals, and all volunteers provided informed consent. A total of 400 (male=108, female= 292) volunteers with RA [1987 revised ACR criteria (Arnett *et al.*, 1988)] were assessed. Of them 8 (6 males) were excluded from the analyses due to missing data for body composition. Data from the remaining 392 [median age: 63.1 (55.5-69.6); median disease duration: 10 (4- 18) years] were analysed.

Assessments

Standing height was measured to the nearest 0.5cm on a Seca 214 Road Rod portable stadiometer (Seca gmbh & co. kg., Hamburg, Germany). Body weight was measured to the nearest 0.1kg. Body composition was assessed by Bioelectrical Impedance, using a Tanita BC-418 MA Segmental Body Composition Analyzer, which incorporates 8 tactile electrodes (Tanita Corporation, Tokyo,

Japan). BMI (kg/m2) was calculated on the basis of measured height and weight. Waist circumference was also measured.

Contemporary disease activity was assessed by ESR, CRP and the DAS (Prevoo *et al.*, 1995). The Anglicised version of the 40-item HAQ (Kirwan and Reeback, 1986) was used to measure physical dysfunction, as a proxy of disease severity. Patients' self-reported smoking status and intensity (i.e. pack-years) were noted.

Data management and analyses

Data were analysed using the Statistical Package for Social Sciences version 15.0 (SPSS Inc. Chicago, IL, USA). Preliminary evaluation of the variables using a Kolmogorov-Smirnov test of normality revealed that none of them required transformation to reach normality. Mean ± standard deviation was calculated for all variables. Differences in BMI, BF and FFM between smoking groups are presented as mean differences with 95% confidence intervals (95% CI).

According to their smoking status, patients were grouped into never-smokers, current smokers and ex-smokers. ANOVA assessed differences in demographic characteristics, BMI and body composition between groups for each gender. ANCOVA was employed to determine whether the differences observed were attributed to smoking status or other confounding factors (e.g. gender, age and disease characteristics).

In the current smokers and ex-smokers groups, further associations between pack-years with BMI and body composition were examined. Thereafter, patients in these groups were divided into quartiles according to pack-years. ANOVA was employed to assess differences in the measured variables between these sub-groups. ANCOVA was used to correct for any confounding factors.

Thereafter, patients were grouped according to a) RA-specific BMI (Stavropoulos-Kalinoglou *et al.*, 2007) and b) gender specific BF (WHO, 2000) thresholds into: underweight, normal-weight, overweight and obese. Subsequently, they were grouped based on gender specific cut-off points for waist circumference (Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults, 2001) into low or high risk, and of FFM into low or normal FFM groups (Schutz *et al.*, 2002). Chi-squared analyses were employed to assess differences between smoking groups in the prevalence of overweight, obesity, high risk and low FFM. For all tests the level of significance was set at $p<0.05$.

5.5.3 Results

Table 14 illustrates means ± standard deviation and the ANOVA results for all studied parameters. Current smokers had significantly lower BMI than ex-smokers (mean differences: male -2.6, 95% CI: -3.5 to -1.7; female: -2.6, 95% CI: -4.8 to -0.5) and never smokers (mean differences: male -1.8, 95% CI: -3 to -0.6; female: -1.4, 95% CI: -2.4 to -0.4). Current smokers also had significantly lower BF compared to ex-smokers (mean difference: male: -4.3, 95% CI: -7.5 to -1.2; female: -3.4, 95% CI: -6.4 to -0.4) and never smokers (mean difference: male: -3.3, 95% CI: -6.3 to -0.4; female: -2.1, 95% CI: -4 to -0.2). FFM did not differ between these groups (mean difference: *current smokers vs ex-smokers* male: -4.6, 95% CI: -10.7 to 1.6; female: -1.2; 95%CI: -3.8 to 1.4; *current smokers vs never smokers* male: -2.7, 95% CI: -9.2 to 3.9; female: 0.1, 95% CI: -2.4 to 2.4). Current smokers had significantly smaller waist circumference than ex-smokers (mean difference: male: -6.2, 95% CI: -10.4 to -1.9; female: -7.8, 95% CI: -13.5 to -2.1) but not never-smokers (mean difference: male: -2.9, 95% CI: -10.6 to 4.9;

female: -3.9, 95% CI: -9.2 to 1.5). Also, ex-smokers had larger waist circumference than never-smokers but the difference was significant only for males (mean difference: male: 3.3, 95% CI: 0.4 to 6.3; female: 3.9, 95% CI: -0.4 to 8.1).

In ANCOVA with gender and smoking as factors, and age, DAS, HAQ and disease duration as covariates, smoking was a significant and independent predictor for BMI ($F_{2, 387}$=8; p<0.001), BF ($F_{2, 387}$=4.4; p<0.05) and waist circumference ($F_{2, 387}$=7.9; p<0.001). Smoking also emerged as a significant predictor of FFM ($F_{2, 387}$=5.1; p<0.05), but inclusion of body mass as a covariate eliminated the effect of smoking on FFM (p>0.05).

There was a significant negative correlation between pack-years and BF (r=-0.46; p<0.001) in the current smokers and the ex-smokers groups. This remained significant after adjustment for gender, age, DAS, HAQ and disease duration ($F_{1, 389}$=4.8; p<0.05). Following pack-year grouping into quartiles (pack-group) ANOVA did not reveal any differences for BMI or body composition among current and ex-smokers pack-groups. However, an ANCOVA model with gender and pack-group as factors and age and weight as covariates (following stepwise elimination of ESR, CRP, DAS, HAQ and disease duration) revealed a significant effect of pack-group on FFM ($F_{3, 217}$=2.7; p<0.05) with heavy smokers exhibiting the lowest values. Mean (95% CI) of this variable in the pack-year sub-groups appear in Figure 10.

Following BMI and BF grouping, chi-squared analyses showed significant differences (p<0.05) in the prevalence of overweight and obese among smoking groups with obesity being more prevalent in ex-smokers (50%), followed by never smokers (39%) and current smokers (30%). Similarly, ex-smokers had

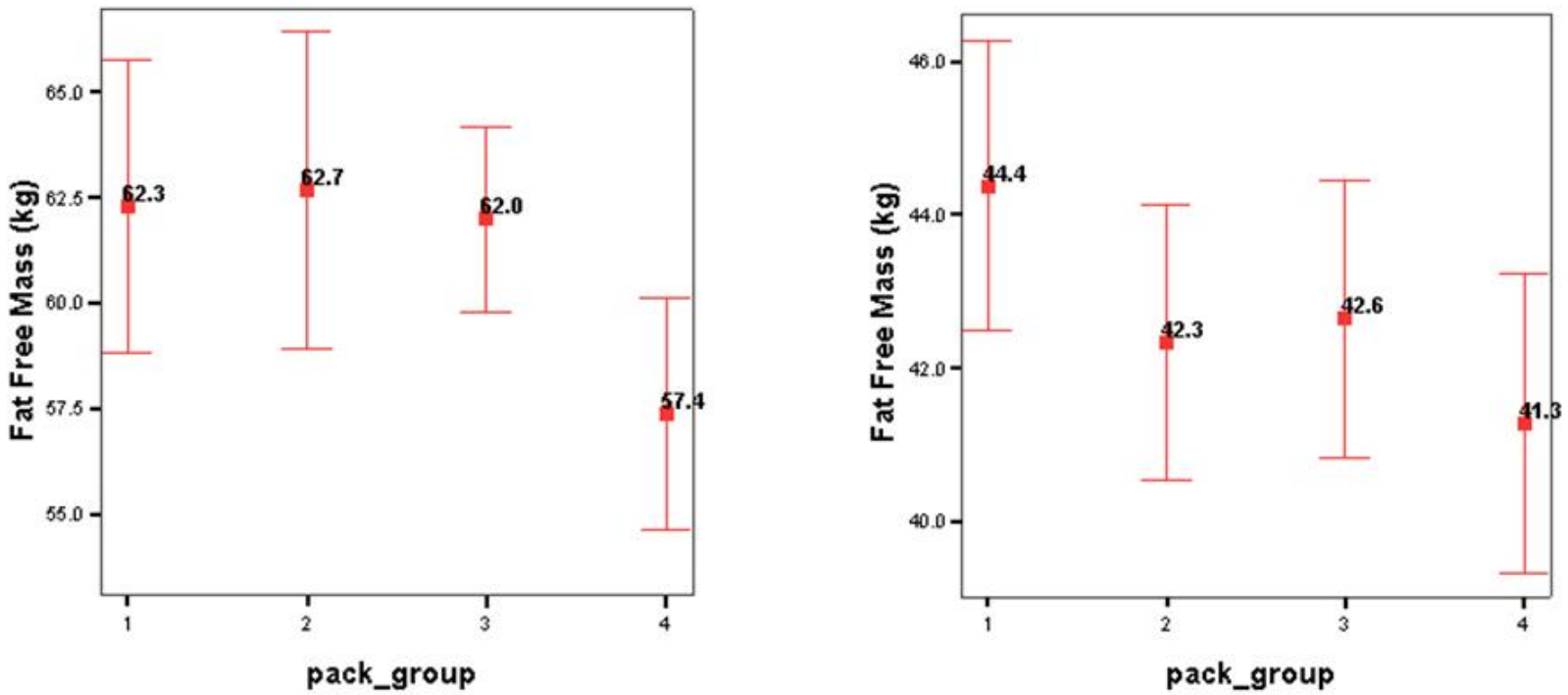
significantly ($p<0.05$) higher prevalence of increased waist circumference (69%) compared to never smokers (60%) and current smokers (49%). However, FFM did not differ between groups ($p>0.05$) (Figure 11).

Table 14: Mean ± standard deviation of measured variables of participants classified as current smokers (CS), ex-smokers (XS) and never-smokers (NS)

Gender	**Male (n=102)**			**Female (n=290)**		
Smoking Status	CS	XS	NS	CS	XS	NS
N	20	50	32	49	97	144
Age (years)	58.8 ±8.1*	65.2 ±9.9†	58.8 ±15	57.4 ±13.3*	64.1 ±11.2†	60.7 ±11.8
Height (cm)	171.3 ±7.1	174.3 ±6.9	172.7 ±7.7	160.9 ±6.9	160.8 ±6.8	159.5 ±6.8
Weight (kg)	76 ±12.9**†	85.8 ±13.6	84.1 ±14.8	67.5 ±14.2*	74.8 ±15.2	69.9 ±13.6
BMI (kg/m^2)	25.8 ±3.3**†	28.4 ±3.8	27.6 ±4.6	26.1 ±5.5*†	28.6 ±5.4	27.5 ±5
BF (%)	24.5 ±6.4**††	28.8 ±6.8	27.8 ±5.6	35.9 ±7*†	39.2 ±6.5	38.1 ±6.7
FFM (kg)	57.2 ±9.4	61.7 ±7.7	59.8 ±10.3	42.5 ±4.8	43.7 ±6.1	42.5 ±6.1
WC (cm)	100 ±7.9**	106.2 ±10.8†	102.9 ±9.3	90.8 ±12.8*	98.6 ±13	94.7 ±12.7
ESR (mm/h)	26.5 ±20.5	22.8 ±21.3	20.7 ±19.7	30.5 ±26	34.3 ±32.7†	25.5 ±19.8
CRP (mg/L)	13.3 ±9.4	16.1 ±20.4	16 ±24.3	21.9 ±23.2†	21.4 ±32.7†	11.9 ±12.5
DAS	4 ±0.9	4.1 ±1.5	3.9 ±1.6	4.5 ±1.5	4.3 ±1.5	4.1 ±1.2
HAQ	0.9 ±0.8	1.4 ±1	1.1 ±0.9	1.5 ±0.9	1.5 ±0.9	1.5 ±0.9
Disease Duration (years)	8.6 ±7.8	11.9 ±10.6	14.6 ±12.7	11.4 ±9.8	13.5 ±10.8	13.5 ±11.1

*Significant difference compared to XS ($p<0.05$)
** Significant difference compared to XS ($p<0.001$)
†Significant difference compared to NS ($p<0.05$)
†† Significant difference compared to NS ($p<0.001$)
BMI= body mass index; BF= body fat; FFM= fat free mass; WC= waist circumference; ESR= erythrocyte sedimentation rate; CRP= C-reactive protein; DAS= disease activity score 28; HAQ= health assessment questionnaire.

Figure 10: Fat free mass for males (Figure 10a) and females (Figure 10b) according to pack-year grouping



Data are presented as means with 95% confidence intervals
Pack-year groups: 1: 1-9 pack years; 2: 10-19 pack years; 3: 20-34 pack years; 4: >35 pack years
*Significant difference compared to group 1 ($p<0.05$)

Figure 11: Prevalence of overweight and obesity, increased waist circumference, and low FFM in smoking groups

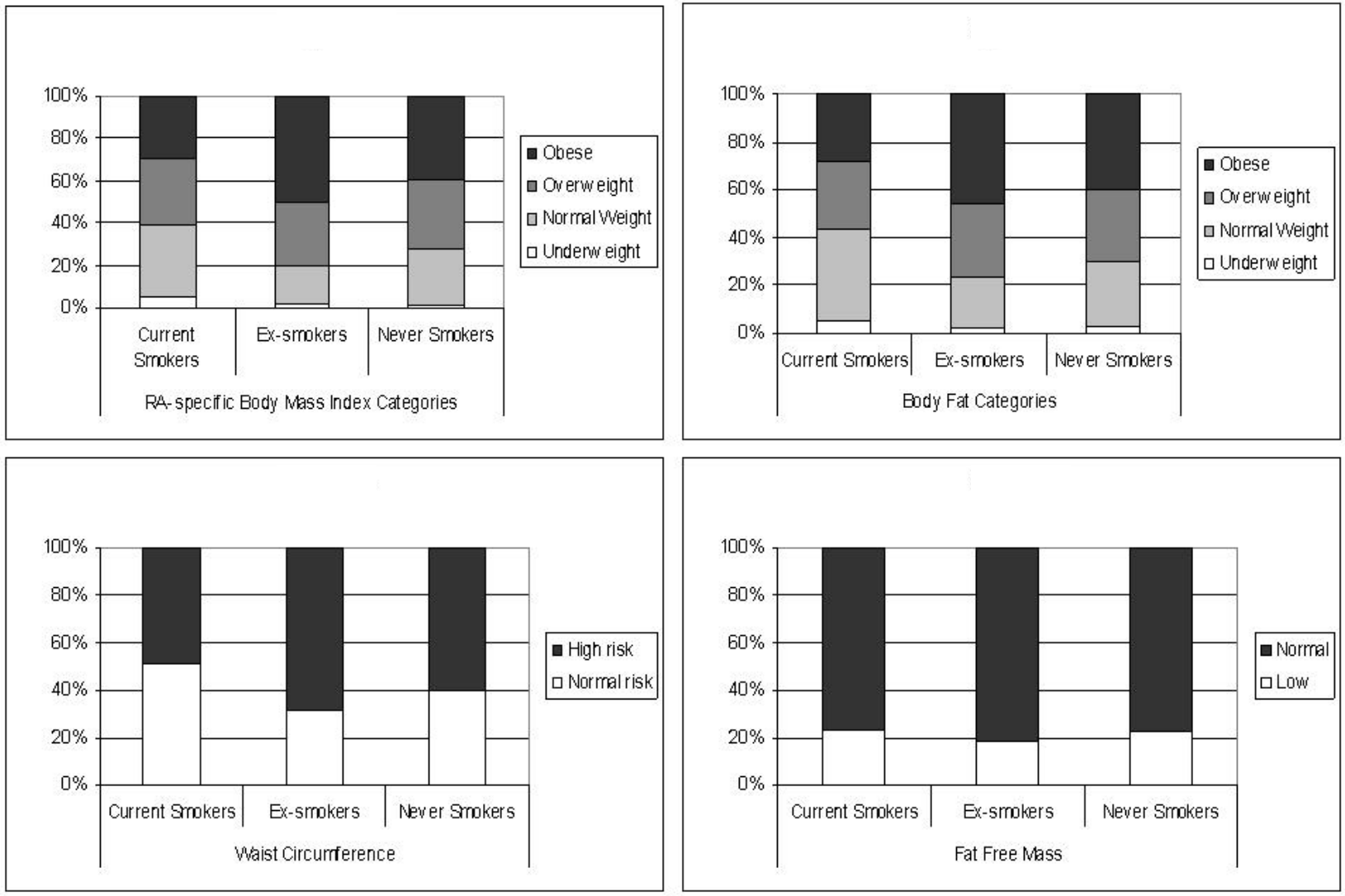


Figure 11a: Prevalence of overweight and obesity based on RA-specific BMI for current-, ex-, and never smokers
Figure 11b: Prevalence of overweight and obesity based on body fat for current-, ex-, and never smokers
Figure 11c: Prevalence of high risk based on waist circumference for current-, ex-, and never smokers
Figure 11d: Prevalence of low fat free mass for current-, ex-, and never smokers
Chi-squared analyses identified significant defences among smoking groups for prevalence of: a) overweight and obesity based on body mass index ($p<0.05$), b) overweight and obesity based on body fat ($p<0.05$) and, c) increased waist circumference ($p<0.05$). Prevalence of low fat free mass did not differ between groups ($p>0.05$).

5.5.4 Discussion

This is the first study to identify significant associations between smoking, body weight and body composition of RA patients: current smokers had significantly lower BMI and BF compared to never-smokers. Both BMI and BF were significantly increased in ex-smokers, whereas very heavy smoking appeared to associate with reduced FFM.

Our observations for BMI are consistent with those in the general population. Both male and female smokers tend to have decreased BMI compared to their non-smoking counterparts (Albanes *et al.*, 1987, Akbartabartoori *et al.*, 2004). In contrast, significant BMI increases have been noted after smoking cessation (Eisenberg and Quinn, 2006). Smokers have increased levels of leptin (Nicklas *et al.*, 1999), which regulates food intake and fat deposition (Klok *et al.*, 2007) and reduced hypothalamic neuropeptide Y (Chen *et al.*, 2006), which regulates appetite (Billington *et al.*, 1991). Smoking-induced increases in the levels of epinephrine, nor-epinephrine and thyroid hormones lead to increased energy expenditure at rest (Collins *et al.*, 1994, Collins *et al.*, 1996) and during light physical activity (Perkins *et al.*, 1989, Perkins, 1992, Walker *et al.*, 1999). However, these effects are short-lived: after smoking cessation, leptin

decreases to levels below those expected for non-smokers of similar weight (Nicklas *et al.*, 1999), while resting energy expenditure (REE) returns to normal (Dallosso and James, 1984).

In RA patients, smoking has been shown to elevate REE (Metsios *et al.*, 2008a); however no data are available on other potential contributors to smoking-related weight-loss or smoking cessation-related weight-gain for this population. Although we did not assess energy intake and expenditure or related regulators (such as leptin), it is likely that the mechanisms behind the reduced body weight of current smokers and the increased body weight of ex-smokers with RA are similar to those described for the general population.

Interestingly, the lower BMI of current smokers in the present study seems to be due to decreased BF rather than FFM. A possible mechanism by which smoking may affect fat metabolism is through reduction in neuropeptide Y. This molecule, not only stimulates food intake, but also promotes white fat lipid storage and decreases brown fat thermogenesis (Billington *et al.*, 1991), so its inhibition through smoking would be expected to have the opposite effects. Additionally, smoking results in decreased adipose tissue lipoprotein lipase (LPL) activity (Chajek-Shaul *et al.*, 1990), which diverts fat storage away from adipose tissue and towards utilization by muscle (Sztalryd *et al.*, 1996), possibly leading to the decreased BF of smokers (Chajek-Shaul *et al.*, 1990, Ferrara *et al.*, 2001). In the present study, the inverse association between smoking and BF appeared to be dose-dependant: increasing pack-years associated with reducing BF levels. Smoking cessation is thought to result in reversal of the mechanisms described above, leading to increases in BF (Chajek-Shaul *et al.*, 1990) and most importantly abdominal fat (Canoy *et al.*, 2005). Indeed, amongst these RA

patients, ex-smokers seemed to be the most “unhealthy” group in terms of body weight and composition, as they exhibited the highest BMI, BF and waist circumference values.

In predominantly healthy people, without muscle-wasting disease, from the general population, smoking of any intensity has been implicated in muscle wasting (Akbartabartoori *et al.*, 2004) by impairing the process of muscle protein synthesis (Petersen *et al.*, 2007). In contrast, in the present study, only very heavy smoking appeared to associate with reduction in FFM. It is possible that the effect of smoking on muscle is of less significance than the muscle loss associated with RA itself, as part of rheumatoid cachexia. This hypothesis is supported by the finding that increased duration of smoking (i.e. pack-years) associated with lower FFM in both current and ex-smokers, which suggests the existence of a threshold below which smoking does not induce further muscle loss in RA patients. A longitudinal study of the impact of smoking intensity (and cessation) on the body composition of patients with RA may throw more light on the mechanistic basis of these observations.

Overall, this study suggests that in RA, smoking associates with reduced body mass and fatness without inducing further muscle loss, except in very heavy smokers; in contrast, smoking cessation associates with increased body mass and fatness. This should not be interpreted as favouring what is a very unhealthy habit. Smoking cessation, even if it occurs in mid-life, reduces most of the later risk of death from tobacco (Boyle *et al.*, 2003). However, smoking cessation is known to result in body weight increase, and this may affect some peoples’ decision to stop smoking (Ferrara *et al.*, 2001, Canoy *et al.*, 2005, Eisenberg and Quinn, 2006). Therefore, any smoking-cessation regimen should be underpinned

by more generalised lifestyle counselling, including advice on exercise and weight management. This is emphasized by the fact that based on recently described RA-specific BMI (Stavropoulos-Kalinoglou *et al.*, 2007), BF (WHO, 2000) and waist circumference thresholds (Grundy *et al.*, 2004) ex-smokers have the highest prevalence of obesity – both total and abdominal. FFM did not differ between groups and the prevalence of low FFM was comparable to that expected in age and gender matched healthy individuals (Schutz *et al.*, 2002).

Within the limitations of this study, it is concluded that RA smokers have lower BMI and BF than RA non-smokers, whilst heavy smokers also have reduced FFM. A history of smoking cessation appears to associate with increases in BMI, BF and waist circumference. Nevertheless, given the numerous adverse effects of smoking on health, RA smokers should be actively advised against it, but smoking cessation programmes should include wider lifestyle counselling for weight control, also focusing on increased physical activity and a healthy diet.

5.6 Association of Habitual Physical Activity, Energy Intake and Inflammation with Body Weight and Composition of Patients with Rheumatoid Arthritis.

5.6.1 Introduction

Obesity is a major health concern in the general population. It associates with several different diseases (Jung, 1997) but most importantly it is an independent predictor for the development of CVD (Hubert *et al.*, 1983). Obesity is caused from a quantitative imbalance between energy intake and expenditure. Physical inactivity and overfeeding are the two main reasons for its development in otherwise healthy individuals (Bray and Bellanger, 2006); genetic characteristics can only enhance or minimise the effects of any lifestyle (Weinsier *et al.*, 1998).

In RA, the pathophysiology of obesity may be more complicated. RA, the most common inflammatory arthritis, is characterised by increased systemic inflammation that leads to joint pain and damage and eventually to physical dysfunction (Arend, 1997). Increased levels of pro-inflammatory cytokines affect energy metabolism and body composition (Roubenoff *et al.*, 1994). RA patients exhibit increased levels of resting energy expenditure (Metsios *et al.*, 2008b); however the excess calories come from muscle breakdown (Roubenoff *et al.*, 1994) leading to reduced muscle mass at the presence of stable body weight, a condition known as rheumatoid cachexia (Rall and Roubenoff, 2004). Ultimately, this results in increased BF content for a given BMI (Stavropoulos-Kalinoglou *et al.*, 2007). Control of inflammation, despite reducing disease activity, does not improve BMI or BF of RA patients (Metsios *et al.*, 2007). This indicates that other stimuli, such as increased physical activity and optimised energy intake are

necessary to initiate calorific expenditure and muscle synthesis. It is however not known whether they can indeed influence that sequence and reduce (or increase) the negative effect of inflammation on body weight and composition of RA patients. The aim of this study was to assess the associations between physical activity, energy intake and inflammation with body weight and composition in patients with RA.

5.6.2 Methods

Participants

Consecutive patients attending routine rheumatology clinics at the Dudley Group of Hospitals NHS Trust, UK, were invited to participate. The study had Local Research Ethics Committee approval by the Dudley Ethics Committee, and all volunteers provided informed consent. A total of 126 (male= 42, female= 84) volunteers with RA [1987 revised ACR criteria (Arnett *et al.*, 1988)] were assessed. Their demographic and disease characteristics appear in Table 15.

Assessments

Standing height was measured to the nearest 0.5cm on a Seca 214 Road Rod portable stadiometer (Seca gmbh & co. kg., Hamburg, Germany). Body composition was assessed by Bioelectrical Impedance, using a Tanita BC-418 MA Segmental Body Composition Analyzer, which incorporates 8 tactile electrodes (Tanita Corporation, Tokyo, Japan). BMI (kg/m^2) was calculated on the basis of measured height and weight. The recently published RA-specific BMI cut-off points (i.e 23kg/m2 for overweight and 28kg/m2 for obesity) were used to categorise patients into underweight, normal-weight, over-weight, or obese(Stavropoulos-Kalinoglou *et al.*, 2007).

Table 15: Demographic, body composition and disease characteristics of participants

	Males	Females
N	42	84
Age (years)	60 (59-64)	59 (55-64)
Disease Duration (years)	7 (4-12)	9 (5-14)
Height (cm)	176 (170-179)	158 (155-161)
Weight (kg)	85.2 (74.9- 92.4)	63.6 (59.7-67.6)
BMI (kg/m^2)	27.5 (24.4-29.6)	25.5 (23.8-27)
BF (%)	26.1 (23.7-30.4)	34.8 (30-40.4)
IPAQ (METmin/week)	2607 (2179-3412)	2369 (1959-2978)
Total Energy intake (kcal/day)	2637.4 (2298.8-2902.2)	2281.2 (1812.4-2506.6)
Carbohydrates (%)	57 (49-60)	59 (55-61)
Fats (%)	24 (19-27)	23 (21-28)
Proteins (%)	19 (16-21)	18 (16-22)
IL-1 (pg/mL)	13.3 (6.1-24.8)	15.5 (11-25.5)
IL-6 (pg/mL)	28.1 (18.3-40.8)	23.7 (11.7-39.3)
TNFα (pg/mL)	19.2 (9.1-26.9)	16.3 (4.7-30.1)
ESR (mm/h)	17 (4-31)	21.0 (6-40)
CRP (mg/L)	18 (11-28)	11.0 (6-24)
DAS	4.4 (2.6-5.3)	3.8 (2.9-5.1)
HAQ	1.4 (0.6-1.8)	1.6 (1-1.9)

BMI: body mass index; BF: body fat; IPAQ: international physical activity questionnaire; IL: interleukin; TNFα: tumour necrosis factor alpha; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; DAS: disease activity scorer 28; HAQ: health assessment questionnaire

The long version of the self-administered international physical activity questionnaire (IPAQ) was utilized to record physical activity. After relevant verbal and written instructions, a simple food diary was distributed to all participants. They were asked to write in detail about all the food and drink they consumed over three non-consecutive days (including a weekend day) during the week before the assessment. Total daily energy intake and percentage carbohydrate, fat and protein intake were calculated using diet analysis software (Recipe Calc 4.0).

Contemporary serological inflammation was assessed by ESR and CRP. The DAS was used to assess clinical disease activity (Prevoo *et al.*, 1995) and the

HAQ to measure functional disability as a proxy for disease severity (Kirwan and Reeback, 1986). Disease duration and smoking habits were recorded from review of the clinical notes. Pro-inflammatory cytokines (i.e. IL-1, IL-6 and TNFα) were assessed in stored serum in batches.

Data Management and Analyses

Data were inserted in a purpose-designed spreadsheet (Microsoft Excel 2003) and audited for accuracy weekly. They were exported for analysis to The Statistical Package for Social Sciences version 15.0 (SPSS Inc. Chicago, IL, USA). The Kolmogorov-Smirnov test of normality was used to assess dispersion of the variables.

The analytic strategy comprised of four steps. The first step was used to assess the associations of BMI and BF as linear variables with physical activity, energy intake, pro-inflammatory cytokines and HAQ [as a surrogate of cumulative disease activity (Provan *et al.*, 2008)] using Spearman's correlations. Results were corrected for variables known to affect BMI and BF in RA [i.e. age, gender (Stavropoulos-Kalinoglou *et al.*, 2007) and smoking (Stavropoulos-Kalinoglou *et al.*, 2008a)]. The second step investigated potential interactions between physical activity, energy intake, pro-inflammatory cytokines and disease characteristics in their association with BMI and BF again as continuous variables. Univariate analyses using either BMI or BF as dependent variables and physical activity, energy intake, pro-inflammatory cytokines and HAQ as covariates were used for that purpose.

The third step of the analyses involved grouping of patients according to their BMI in underweight, normal-weight, overweight and obese. ANOVA was used to trace differences between groups for physical activity, energy intake, pro-

inflammatory cytokines and HAQ. Results were again standardised for age, gender and smoking using analysis of ANCOVA. Binary logistic models were devised to identify which of the variables: IPAQ, energy intake, pro-inflammatory cytokines or HAQ, associated closer with obesity or underweight. Age, gender and smoking were again included in the initial models, however, following elimination of insignificant variables, only those that associated significantly qualified for the final model.

The fourth and final step of the analyses involved re-grouping of the patients this time according to gender specific BF cut-off points (WHO, 2000) in underfat, normal-fat, overfat and obese. Precisely the same analyses as in step three were conducted. Namely: ANOVA for differences between groups in IPAQ, energy intake, pro-inflammatory cytokines and HAQ; ANCOVA to standardise the results for age, gender and smoking; binary logistic to assess which of the variables (IPAQ, energy intake, pro-inflammatory cytokines or HAQ) associated closer with obesity or underfat.

Dispersion of data is reported as median (interquartile range). Results of the logistic models are reported as odds ratios with 95% confidence intervals (OR, 95% CI). Statistical significance was set at $p<0.05$.

5.6.3 Results

Step 1

BMI inversely correlated with IPAQ (r=-0.511, p=0.000) and positively with energy intake (r=0.331, p=0.016) and HAQ (r=0.133, p=0.042). BF inversely correlated with IPAQ (r=-0.575, p=0.000) and positively with HAQ (r=0.201, p=0.037). Neither BMI nor BF correlated with any of the cytokines. These associations were independent of any potential confounding factors.

Step 2

When the interactions of IPAQ, energy intake, cytokines and HAQ were examined, univariable analyses indicated that BMI associated with IPAQ ($F_{1, 125}$= 12.01; p=0.001) and total energy intake ($F_{1, 125}$= 6.624; p=0.014), whereas BF only with IPAQ ($F_{1, 125}$= 11.858; p=0.001). The previous observed associations of BMI and BF with HAQ were lost and cytokines again did not associate with either of the dependent variables.

Step 3

Normal-weight patients were more physically active compared to both overweight (p=0.006) and obese (p=0.000) whereas underweight patients consumed significantly less calories compared to all other patients (p>0.05 in all case; Figure 12a and 12b). Normal weight patients had lower HAQ scores compared to all other participants however the differences were not statistically significant. Levels of pro-inflammatory cytokines did not differ between groups. The above results were independent of any possible confounders. Binary logistic models indicated that among the tested variables, IPAQ was the sole predictor of obesity (OR=0.988, 95% CI: 0.982-0.996; p=0.023). Patients in the lowest IPAQ quartile were six times more likely to be obese (OR=6.0, 95% CI: 4.432- 8.991; p=0.001) and those in the second lowest 4.5 (OR= 4.571, 95% CI= 2.903- 6.012; p=0.032) compared to those in the highest IPAQ quartile. On the contrary, energy intake was the only predictor for underweight (OR=0.990, 95% CI: 0.982-0.999; p=0.025).

Step 4

Normal-fat patients were significantly more physically active compared to both over-fat (p=0.004) and obese (p=0.000) whereas the underfat consumed significantly less calories compared to overweight (p=0.028) and obese participants (p=0.000; Figure 12c and 12d). Again normal-weight patients tended to have lower HAQ but the differences between groups were not statistically significant. Cytokines again did not differ between groups. Following corrections for age, gender and smoking, the observations for physical activity persisted. However, following corrections for smoking (alone and in combination with age and gender) the difference in energy intake between groups was nonexistent ($F_{1,125}$= 2.114; p=0.112). In the binary logistic models, where the interaction of physical activity, energy intake and inflammation was tested, only IPAQ was found to associate with obesity (OR=0.991, 95% CI: 0.983-0.997; p= 0.008). Patients in the lowest quartile of IPAQ were >7 times more likely to be obese (OR=7.562, 95% CI: 4.499-12.152; p=0.004) and those in the second lowest 5.5 (OR=5.500, 95% CI: 2.145-8.412; p=0.033). None of the variables in question (i.e. physical activity, energy intake and inflammation) were able to predict underfat.

Figure 12: Differences in IPAQ and energy intake between BMI and BF groups

Figure 12a: Differences in IPAQ between BMI groups

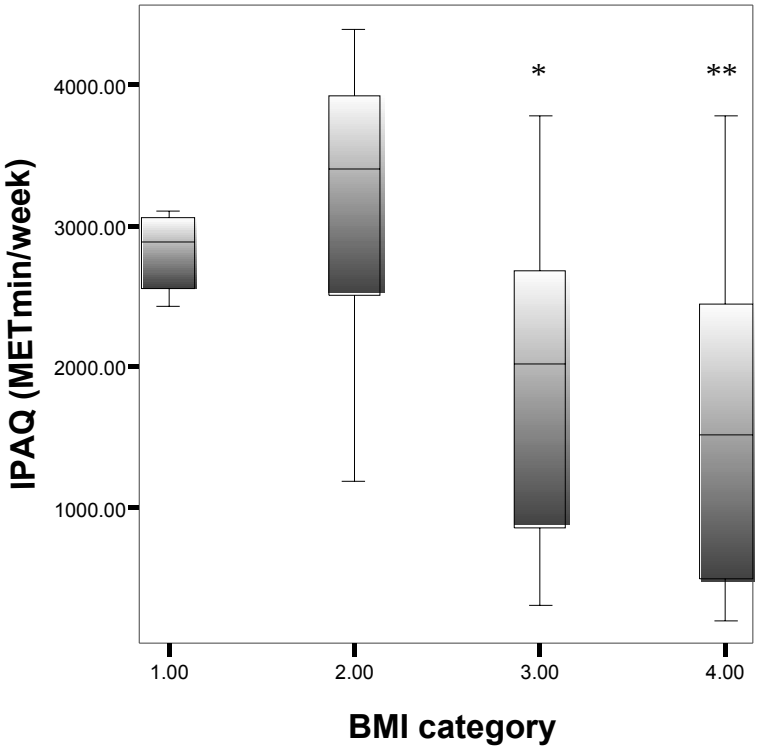


Figure 12b: Differences in energy intake between BMI groups

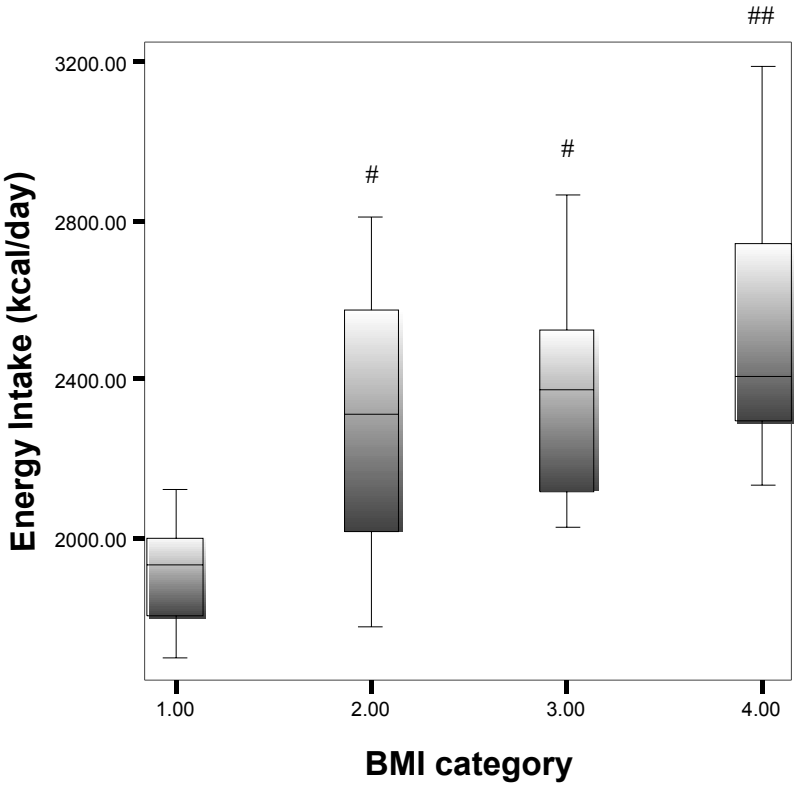


Figure 12c: Differences in IPAQ between BF groups

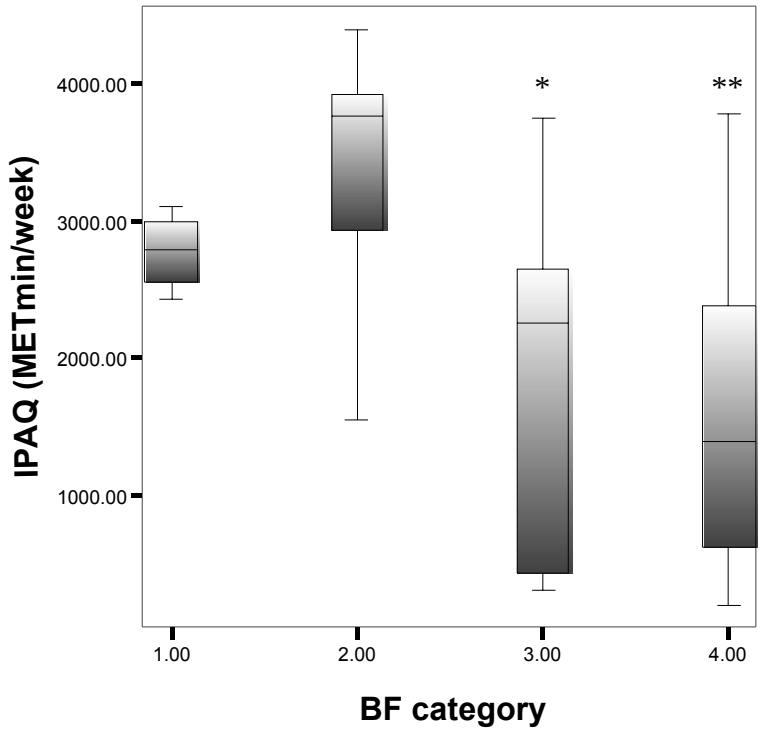
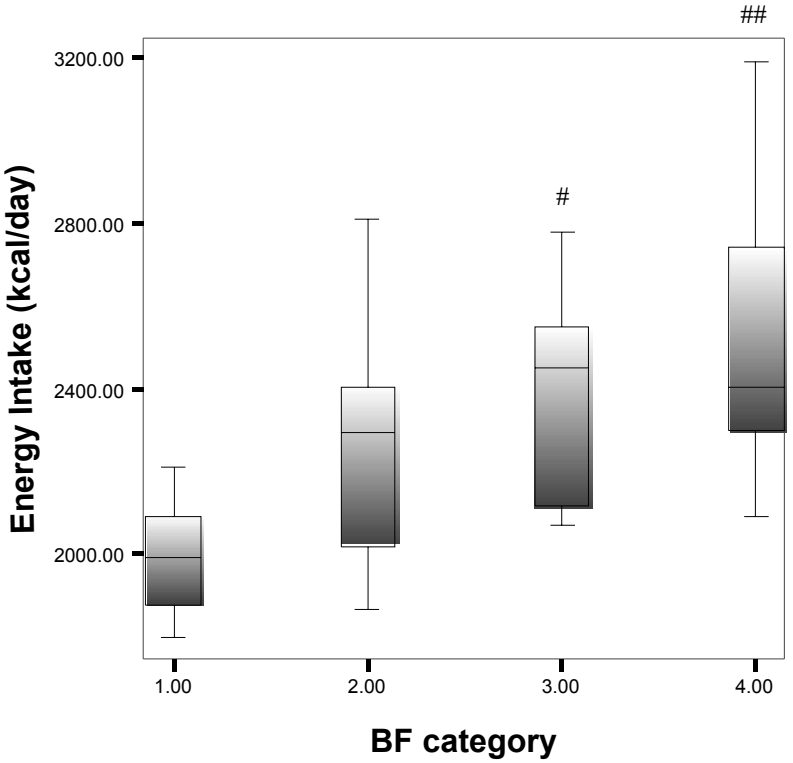


Figure 12d: Differences in energy intake between BF groups



* Significant difference with normal weight or normal fat (p<0.05)
** Significant difference with normal weight or normal fat (p<0.001)
# Significant difference with underweight or underfat (p<0.05)
Significant difference with underweight or underfat (p<0.001)

5.6.4 Discussion

This is the first study to assess possible interactions between habitual physical activity, diet and inflammation in their associations with body weight and composition in RA patients. Our results indicate that increased habitual physical activity associated with reduced BMI and BF; physically active patients were less likely to be overweight or obese compared to their inactive counterparts. Energy intake on the other hand did not differ between normal-weight, overweight and obese patients. However, underweight patients consumed significantly less calories compared to other participants even though their activity levels were comparable. Inflammation did not differ between the patients and body weight classification was not affected by it.

In individuals free from any metabolic disorders, body weight and composition depend almost exclusively on lifestyle (Bray *et al.*, 1998). When energy needs are met by energy supply, body weight is maintained. Disturbances in this balance can cause weight gains or losses. Increasing physical activity or over-, under-feeding are the main such disturbances. Overall, physical activity reduces body weight by burning away BF while preserving or slightly increasing muscle mass. Overfeeding results in increased body weight mainly by increasing BF; underfeeding results in reduced body weight from concomitant decreases in both BF and muscle mass (in a ratio of about 3:1) (Bray *et al.*, 1998).

The mechanisms leading to weight gain or loss in RA seem to be the same as in the general population: imbalance between energy intake and expenditure. Inflammation as measured via the cytokines did not seem to affect BMI or BF. The main reason for that is the fluctuation in disease activity. While changes in body composition occur over a long period of time (Bray *et al.*, 1998) disease activity,

and with it inflammation, can vary enormously within very short periods. However, the chronic inflammatory load of the disease, as reflected in the HAQ (Provan *et al.*, 2008), seemed to associate both with BMI and BF in our initial analyses. When IPAQ was introduced as a confounder though, these associations were lost. It seems that physical activity, irrespective of the severity of the disease can have beneficial effects on body weight and composition of RA patients. This is in line with current evidence suggesting that exercise is helpful for all RA patients (Metsios *et al.*, 2008c) and it doesn't cause any further damage to the joints (de Jong *et al.*, 2004).

Even if inflammation didn't show up as a significant predictor of either BMI or BF in our study, it is known to significantly affect body composition of RA patients. During periods of high disease activity, hypersecretion of cytokines – especially of IL-1, IL-6 and TNFα – enhance muscle wasting (Arshad *et al.*, 2007). TNFα is known to activate nuclear factor kappa-beta dependent processes which can both enhance proteinolysis (Li *et al.*, 2003) and inhibit proteinosynthesis (Langen *et al.*, 2001) in the muscle. TNFα also reduces insulin action in the muscle (Hotamisligil *et al.*, 1994); this results in limited transportation through muscle-cell membrane of amino acids essential for proteinosynthesis (Rall and Roubenoff, 2004). IL-1 and -6 have an additive effect to that of TNFα in muscle catabolism. The exact mechanism by which they do that is not clear; however inhibition of both IL-1 and TNFα better prevents muscle wasting than inhibition of TNFα alone (Roubenoff *et al.*, 2002). During periods of less active disease, these processes are decelerated (Arshad *et al.*, 2007).

However, in order for the muscle to initiate its anabolic processes an adequate stimulus needs to be present (McArdle *et al.*, 2001). Physical activity,

even at very low levels, is probably the best such stimulus. Low-intensity aerobic exercise is known to acutely and chronically improve insulin action on the muscle allowing for greater amino-acid delivery which can then be used to built protein (Fujita *et al.*, 2007). Resistance exercise initiates muscle reconstruction mechanisms, mainly through activation of the "mammalian target of rapamycin" signalling pathway (Kubica *et al.*, 2005). Energy expenditure is also increased both during as well as for several hours following exercise (Poehlman, 1989, Stavropoulos-Kalinglou, 2002). This excess energy is mainly supplied by stored fat (Holloszy and Kohrt, 1996) and eventually results in reductions in BF (Bray *et al.*, 1998). Nutrition also plays a significant role in these changes in body composition. In order for the exercise-induced muscle synthesis to occur, diet needs to provide adequate supply of amino-acids (Hebuterne *et al.*, 2001). On the contrary, in order for someone to lose weight, energy intake should be lower than the energy demands. Even if energy intake and expenditure are balanced, exercise can stray nutrient storage away from BF and towards glycogen; coupled with the increases in muscle mass, this results in stable total body weight but reduced BF (Holloszy and Kohrt, 1996). This is also the most likely explanation for the association of energy intake with underweight (as indicated by low BMI) but not with underfat (as indicated by low BF). In a disease that is associated with muscle wasting, underfeeding could result in accelerated muscle depletion leading to decreased overall weight but not BF (Roubenoff *et al.*, 1994).

The design of our study does not allow us to draw safe conclusions about the sequence at which the above described processes occur. However, the most likely path by which inflammation, physical activity and diet affect body composition is the following: during active disease inflammatory processes reduce

muscle mass. Physical activity during these periods is lowered (Metsios *et al.*, 2008c) and generally patients consume less calories (Metsios *et al.*, 2007). During periods of less active disease, physically active patients provide adequate stimuli for their muscles to initiate reconstructive processes and also expend enough energy to reduce their body weight/fat. Those who do not meet their energy needs with their diet continue to lose body weight and muscle mass.

However, the main question still remains, how much exercise is enough? Only the most active of our participants, achieved activity levels comparable to those reported for the general population (Rütten *et al.*, 2003). However, both in terms of calories and of nutrient composition, their diet was well balanced (apart from underweight) (Krauss *et al.*, 2000). From our data, it seems that even very low levels of physical activity when accompanied by a balanced diet are adequate to maintain a desirable BMI and BF. This is in agreement with observations made in the general population. Individuals who exhibit moderate levels of habitual physical activity throughout their lives have significantly lower BMI compared to those with low levels of habitual physical activity or even those who engage in opportunistic exercise (Wareham *et al.*, 2005). We need to mention though that some studies have investigated the effects of exercise on aspects of body composition in RA and their conclusions were in favour of intensive exercise (de Jong *et al.*, 2003, Marcora *et al.*, 2005b). Even though, such observations are extremely useful in the design of exercise interventions, more basic counselling focusing on increasing habitual physical activity is equally important. Our observation on the balanced diet of RA patients is not new (Arshad *et al.*, 2007). It is the first time though, that reduced energy intake has been associated with underweight status in this population. In a similar line are the data of Marcora *et*

al. (Marcora *et al.*, 2005a) suggesting that increased protein intake can reverse cachexia in RA patients.

In conclusion, increased levels of habitual physical activity associate with reduced BMI and BF. Most likely, during periods of reduced disease activity, such physical activity is able to increase muscle mass and reduce body fat. Energy intake, even though generally well balanced in RA patients, is a major determinant of underweight. Given the adverse effect of obesity and its high prevalence in RA, patients should be advised towards a more active lifestyle. However, exercise professionals should always take into account the movement limitations of these patients. Those with increased levels of inflammation need to consume protein-rich diet in order to prevent excessive muscle loss. Further research investigating the suitability of exercise modalities and diet, and the resulting effects on RA is advocated.

Chapter 6: General Discussion

The aim of the current PhD work was to investigate aspects of obesity in patients with chronic inflammatory diseases, and especially RA. Specifically, this project focused on the definition of obesity, its associations with RA characteristics and CVD risk as well as the significant contributions of lifestyle, and especially physical activity, in its development. The main findings of this study expanded our understanding on selected parameters of obesity in RA and emphasised the significance of physical inactivity in the development of it.

As highlighted in the literature review, obesity in this population has attracted minimal scientific and clinical attention. The few studies that have addressed this issue have used BMI cut-off points devised for the general public (i.e. 25kg/m^2 for overweight and 30kg/m^2 for obesity). However, as indicated in the first study of this project, the use of these cut-offs in RA patients is incorrect. These patients, exhibit significantly increased levels of BF compared to patients without wasting diseases, such as OA, and, more importantly, compared to healthy controls of the same BMI. This has led to the redefinition of overweight and obesity for RA patients (i.e. 23kg/m^2 for overweight and 28kg/m^2 for obesity); the new RA-specific BMI cut-off points are able to better identify individuals with increased BF (Stavropoulos-Kalinoglou *et al.*, 2007).

Proper classification of adiposity is important in both the management of RA as well as the prevention of relevant health conditions and most importantly CVD. The second study of the present project identified a significant association of BMI with disease activity. Interestingly, both underweight and obese patients exhibited worse disease characteristics compared to their normal-weight

counterparts. Furthermore, the third study found significant association of BMI with classical CVD risk factors. As expected, CVD profile deteriorated with increasing BMI. The significant finding of this study however, was the BMI at which CVD risk, as indicated by the risk factors, increased. Patients with one risk factor had a median BMI of <25kg/m^2 whereas those with the MetS <30 kg/m^2 and by applying general BMI thresholds would be classified as normal-weight and overweight respectively; however, based on RA-specific BMI thresholds they would be classified as over-weight or obese. This could be important in routine clinical practice, where such classifications may be used to target patients at increased risk for screening, early identification and management of risk factors (WHO, 2000).

However, adiposity does not affect all individuals in the same way. The fourth study of this project was able to identify normal-weight individuals with a metabolic phenotype typically related to obesity and others who, despite being obese, exhibited a metabolic phenotype related to normal-weight. These distinct subtypes of the population also exhibit altered inflammatory profiles, thus their identification might have significant clinical implications for the treatments they receive.

The final two studies of this project were able to identify factors that influence obesity, both in terms of BMI and BF, in patients with RA. Smoking, physical activity and to a lesser extent diet were found to exert significant effects on BMI and BF. Smoking was found to have a significant protective effect against obesity while smoking cessation associated with significantly increased adiposity. This however should not be interpreted as favouring a generally unhealthy habit. Smoking cessation, an effective means of reducing smoking related health risks,

should be accompanied by weight management in patients with RA. Physical activity also had a protective effect against obesity. Physically active patients had, on average, lower BMI and were less likely to be obese compared to their physically inactive counterparts. Energy intake on the other hand was not associated with increased but rather with decreased BMI. Underweight patients consumed significantly less calories compared to the rest participants. The most important finding of this study however, was probably the lack of association between inflammation and any aspect of adiposity. The weak associations of disease severity with BMI and BF were eliminated as soon as physical activity was introduced in the statistical models. This on its own provides a very strong justification for the use of exercise in the prevention and treatment of obesity in RA patients. Such interventions also have the potential to reduce CVD risk (Metsios *et al.*, 2008c) and to improve physical function without aggravating the disease or causing any further joint damage (de Jong *et al.*, 2003, de Jong *et al.*, 2004). These observations, coupled with the potential direct benefits of exercise on inflammation (reviewed in Handschin and Spiegelman, 2008) underscore the importance of exercise in the management of RA.

Even though this project did not assess any possible interventions to counteract obesity, it is a very useful tool in the hands of health or exercise professionals who might want to do so. Over all, the present project has highlighted the significant impact of obesity on health of RA patients. This has provided a strong rational for its treatment, something that was lacking until now. Having assessed the factors that influence obesity, it has also provided definite directions for future research protocols or clinical interventions with exercise appearing to be the most promising modality for weight control in these patients.

Chapter 7: Limitations

Even though the methodology of the present project was carefully designed, several potential limitations might have influenced our findings. Depending on whether they affect the whole project or individual studies, limitations are listed below as general or study specific respectively.

7.1 General

- The cross-sectional nature of the observations: the associations found are interesting and can serve for hypothesis generation, but they do not provide definitive evidence for causality or directionality, which can only be addressed in long-term prospective studies.
- Body composition was assessed by bioelectrical impedance. This method, has been validated (Gray *et al.*, 1989, Oppliger *et al.*, 1992, Tanaka *et al.*, 1999, Bolanowski and Nilsson, 2001, Demura *et al.*, 2004) and is thought to be suitable for body composition studies in diverse populations (Bolanowski and Nilsson, 2001, Lofthouse *et al.*, 2002, Demura *et al.*, 2004, Demura *et al.*, 2005). It correlates well with the "gold standards" of dual-energy x-ray absorptiometry and hydrostatic weighing (Demura *et al.*, 2004) and is widely used in RA research (Lemmey *et al.*, 2001, Lofthouse *et al.*, 2002, Stavropoulos-Kalinoglou *et al.*, 2007, Stavropoulos-Kalinoglou *et al.*, 2008a, Stavropoulos-Kalinoglou *et al.*, 2008b) (Metsios *et al.*, 2007, Metsios *et al.*, 2008b), but it has not actually been specifically validated in the RA population.

7.2 Study Specific

- **Study 2:** The presence of erosions was assessed only qualitatively in radiographs of the hands and wrists: this does not allow quantitative analysis as all methods for quantification of erosions require x-rays from several different joints. Thus, patients with erosive disease were grouped together irrespective of the extent of erosive damage, and severity of joint damage could only be inferred by joint surgery. However, we have included these measures only as simple indications and we do not draw any of our major conclusions from them.
- **Study 4:** The prospective, longitudinal branch of this study had a very small number of participants and it cannot be used to draw definite conclusions. Comparisons with the general population are based on the existing literature and not on a control group. Thus, the mechanisms described in the study are based entirely on the available literature and not on our assessments. On the other hand, the size of the cohort and the prospective collection of data in a standardised, systematic manner are important strengths, as they minimised missing values and selection bias.
- **Study 5:** Although self-report of smoking, especially smoking history, is generally reliable, both under- and over-reporting can occur (Fendrich *et al.*, 2005). This is unlikely to have influenced the primary findings of this study, i.e. the differences between current-, ex-, and non-smokers, while any miss-reporting in pack-years may have been smoothed by the large number of participants. It is difficult to assess any other selection bias: the prevalence of current-, ex- and non-smokers among the participants of this study was similar to that reported for local general population subjects of similar age (General

Household Survey 2005, 2006), although it was different to another RA cohort established more than 10 years ago (Saag *et al.*, 1997).

- **Study 6:** The methods used to assess energy intake and expenditure depend on self-reported information. Even though widely used in research and extensively validated (Craig *et al.*, 2003), IPAQ has not been tested for RA patients; on the other hand, individuals who consume excessive amounts of food are known to underreport their energy intake in food diaries (Heitmann and Lissner, 2005).

Chapter 8: Suggestions for Future Research

This project is the first systematic approach to obesity in patients with chronic inflammation, and especially RA and also the first to propose a more significant contribution of physical activity, compared to inflammation, in obesity of these patients. It has viewed this population as a group of individuals with different needs from the general population and was thus able to focus on their specific needs. Overall, studies confirming the findings of the present project, especially if using improved methodology that eliminated the limitations, are advocated.

The first study proposed lower BMI thresholds for overweight and obesity and the following studies proved them to be more accurate for the identification of patients at risk in this population. A re-examination of existing cohorts and other interventional studies based on the newly proposed RA-specific BMI cut-off points might increase our understanding of obesity in this population. The findings of the second and third studies need to be confirmed in longitudinal investigations. Cohorts assessing the change in BMI and BF over a period of years and the resulting changes in RA and CVD characteristics are highly useful as are those with death as their endpoint.

In terms of scientific potential, the findings of the fourth study are very intriguing. Differences in white and brown adipose tissue, their vascularisation and the effects of weight gain or loss are largely unknown even in the general population. The fourth study of the present project has proposed a theory by which increasing adiposity might affect RA and CVD profile in some patients but not in others. This theory remains to be tested in clinical and basic investigations.

The results of such experiments will most likely provide useful information for the general population as well.

In terms of clinical application the final two studies have probably provided the most interesting information. The association of smoking cessation with increases in adiposity is worrying. If confirmed in longitudinal studies, weight management programmes that will be used in conjunction to smoking cessation should be developed. This is probably the greatest challenge for researchers that will decide to continue the steps of this project. As discussed in the final study, exercise seems to be the most likely method that will induce weight loss, or prevent weight gain, in RA patients. Due to the manifestations of the disease, (i.e. joint pain, swelling, and stiffness) and limited mobility of the patients, exercise professionals need to adapt their methods to the individual needs of each patient. Also the frequency and severity of flares related to RA need to be taken into account and incorporated into the programme so that the benefits gained through exercise are not lost due to inactivity.

Finally, even though not among the main questions of the present project, an association between poor diet and underweight was observed. Due to the significant health effects of underweight, identified both in this project and in several other studies, this association should receive some further attention. Dietary modification, possibly in combination with exercise, especially resistance training, could improve the status of these individuals.

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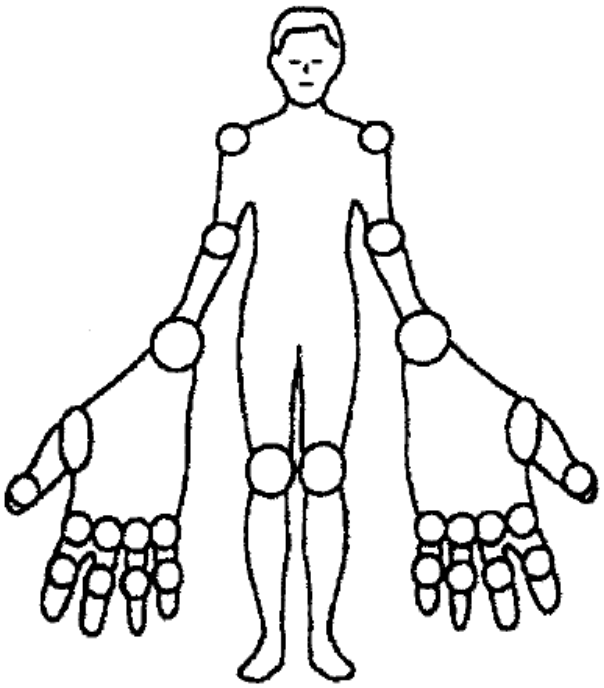
Appendix 1: Disease Activity Score 28

Please mark on the line above how your overall health has been in the last week

Worst ever health **Best ever health**

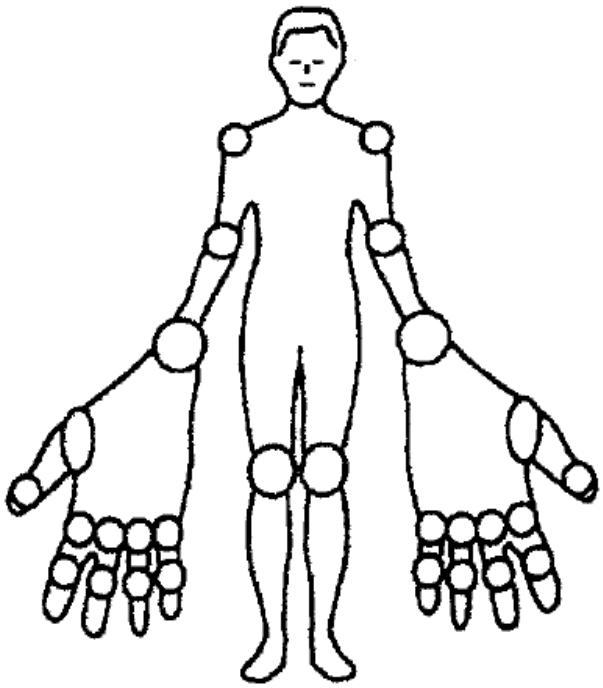
28 Joint Count

Swollen Joints



Total…….

Tender joints



Total…….

Appendix 2: Health Assessment Questionnaire

Name: _____ Date: _____

Please place an "x" in the box which best describes your abilities OVER THE PAST WEEK:

	WITHOUT ANY DIFFICULTY	WITH SOME DIFFICULTY	WITH MUCH DIFFICULTY	UNABLE TO DO
DRESSING & GROOMING				
Are you able to:				
Dress yourself, including shoelaces and buttons?	☐	☐	☐	☐
Shampoo your hair?	☐	☐	☐	☐
ARISING				
Are you able to:				
Stand up from a straight chair?	☐	☐	☐	☐
Get in and out of bed?	☐	☐	☐	☐
EATING				
Are you able to:				
Cut your own meat?	☐	☐	☐	☐
Lift a full cup or glass to your mouth?	☐	☐	☐	☐
Open a new milk carton?	☐	☐	☐	☐
WALKING				
Are you able to:				
Walk outdoors on flat ground?	☐	☐	☐	☐
Climb up five steps?	☐	☐	☐	☐

Please check any AIDS OR DEVICES that you usually use for any of the above activities:

☐ Devices used for Dressing (button hook, zipper pull, etc.)
☐ Built up or special utensils
☐ Crutches
☐ Cane
☐ Wheelchair
☐ Special or built up chair
☐ Walker

Please check any categories for which you usually need HELP FROM ANOTHER PERSON:

☐ Dressing and grooming ☐ Arising ☐ Eating ☐ Walking

Please place an "x" in the box which best describes your abilities OVER THE PAST WEEK:

	WITHOUT ANY DIFFICULTY	WITH SOME DIFFICULTY	WITH MUCH DIFFICULTY	UNABLE TO DO
HYGIENE				
Are you able to:				
Wash and dry your body?	☐	☐	☐	☐
Take a tub bath?	☐	☐	☐	☐
Get on and off the toilet?	☐	☐	☐	☐
REACH				
Are you able to:				
Reach and get down a 5 pound object (such as a bag of sugar) from above your head?	☐	☐	☐	☐
Bend down to pick up clothing from the floor?	☐	☐	☐	☐
GRIP				
Are you able to:				
Open car doors?	☐	☐	☐	☐
Open previously opened jars?	☐	☐	☐	☐
Turn faucets on and off?	☐	☐	☐	☐
ACTIVITIES				
Are you able to:				
Run errands and shop?	☐	☐	☐	☐
Get in and out of a car?	☐	☐	☐	☐
Do chores such as vacuuming or yard work?	☐	☐	☐	☐

Please check any AIDS OR DEVICES that you usually use for any of the above activities:

☐ Raised toilet seat
☐ Bathtub bar
☐ Long-handled appliances for reach
☐ Bathtub seat
☐ Long-handled appliances in bathroom
☐ Jar opener (for jars previously opened)

Please check any categories for which you usually need HELP FROM ANOTHER PERSON:

☐ Hygiene
☐ Reach
☐ Gripping and opening things
☐ Errands and chores

Your ACTIVITIES: To what extent are you able to carry out your everyday physical activities such as walking, climbing stairs, carrying groceries, or moving a chair?

COMPLETELY	MOSTLY	MODERATELY	A LITTLE	NOT AT ALL
☐	☐	☐	☐	☐

Your PAIN: How much pain have you had IN THE PAST WEEK?
On a scale of 0 to 100 (where zero represents "no pain" and 100 represents "severe pain"), please record the number below.

Your HEALTH: Please rate how well you are doing on a scale of 0 to 100 (0 represents "very well" and 100 represents "very poor" health), please record the number below.

Appendix 3: International Physical Activity Questionnaire

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ
The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ
Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation
Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ
International collaboration on IPAQ is on-going and an International Physical Activity Prevalence Study is in progress. For further information see the IPAQ website.

More Information
More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). Assessment of Physical Activity: An International Perspective. Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the vigorous and moderate activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

☐ Yes

☐ No → Skip to PART 2: TRANSPORTATION

The next questions are about all the physical activity you did in the last 7 days as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, heavy construction, or climbing up stairs as part of your work? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ days per week

☐ No vigorous job-related physical activity → Skip to question 4

3. How much time did you usually spend on one of those days doing vigorous physical activities as part of your work?

_____ hours per day
_____ minutes per day

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads as part of your work? Please do not include walking.

_____ days per week

☐ No moderate job-related physical activity → Skip to question 6

5. How much time did you usually spend on one of those days doing moderate physical activities as part of your work?

_____ hours per day
_____ minutes per day

6. During the last 7 days, on how many days did you walk for at least 10 minutes at a time as part of your work? Please do not count any walking you did to travel to or from work.

_____ days per week

☐ No job-related walking → Skip to PART 2: TRANSPORTATION

7. How much time did you usually spend on one of those days walking as part of your work?

_____ hours per day
_____ minutes per day

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the last 7 days, on how many days did you travel in a motor vehicle like a train, bus, car, or tram?

_____ days per week

☐ No traveling in a motor vehicle → Skip to question 10

9. How much time did you usually spend on one of those days traveling in a train, bus, car, tram, or other kind of motor vehicle?

_____ hours per day
_____ minutes per day

Now think only about the bicycling and walking you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the last 7 days, on how many days did you bicycle for at least 10 minutes at a time to go from place to place?

_____ days per week

☐ No bicycling from place to place → Skip to question 12

11. How much time did you usually spend on one of those days to bicycle from place to place?

_____ hours per day
_____ minutes per day

12. During the last 7 days, on how many days did you walk for at least 10 minutes at a time to go from place to place?

_____ days per week

☐ No walking from place to place → Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

13. How much time did you usually spend on one of those days walking from place to place?

_____ hours per day
_____ minutes per day

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the last 7 days in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, chopping wood, shoveling snow, or digging in the garden or yard?

_____ days per week

☐ No vigorous activity in garden or yard → Skip to question 16

15. How much time did you usually spend on one of those days doing vigorous physical activities in the garden or yard?

_____ hours per day
_____ minutes per day

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate activities like carrying light loads, sweeping, washing windows, and raking in the garden or yard?

_____ days per week

☐ No moderate activity in garden or yard → Skip to question 18

17. How much time did you usually spend on one of those days doing moderate physical activities in the garden or yard?

_____ hours per day
_____ minutes per day

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate activities like carrying light loads, washing windows, scrubbing floors and sweeping inside your home?

_____ days per week

☐ No moderate activity inside home → Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY

19. How much time did you usually spend on one of those days doing moderate physical activities inside your home?

_____ hours per day
_____ minutes per day

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the last 7 days solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the last 7 days, on how many days did you walk for at least 10 minutes at a time in your leisure time?

_____ days per week

☐ No walking in leisure time → Skip to question 22

21. How much time did you usually spend on one of those days walking in your leisure time?

_____ hours per day
_____ minutes per day

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do vigorous physical activities like aerobics, running, fast bicycling, or fast swimming in your leisure time?

_____ days per week

☐ No vigorous activity in leisure time → Skip to question 24

23. How much time did you usually spend on one of those days doing vigorous physical activities in your leisure time?

_____ hours per day
_____ minutes per day

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis in your leisure time?

_____ days per week

☐ No moderate activity in leisure time → Skip to PART 5: TIME SPENT SITTING

25. How much time did you usually spend on one of those days doing moderate physical activities in your leisure time?

_____ hours per day
_____ minutes per day

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the last 7 days, how much time did you usually spend sitting on a weekday?

_____ hours per day
_____ minutes per day

27. During the last 7 days, how much time did you usually spend sitting on a weekend day?

_____ hours per day
_____ minutes per day

This is the end of the questionnaire, thank you for participating.

Appendix 4: Food Diary

FOOD DIARY

Name:

Date: Week of Treatment:

Participant Code:

With this diary we will record your every day food consumption.
We need you to describe **in every detail**:

1. Exactly what you eat and drink (e.g. chicken breast without skin, semi-skimmed milk).
2. How much of each ingredient you use (in household measures such as teaspoons or tablespoons; glasses; small, medium or large portions etc.).
3. And how you cook it (e.g. fried, in the oven).

Example of one day food diary:

Breakfast	Lunch	Dinner	Snacks	Water
1 glass of semi-skimmed milk	1 fish fillet fried	1 medium portion of spaghetti with canned tomato sauce and garlic	2 slices of white medium sliced bread with two teaspoons of butter	8 glasses
2 fried eggs medium size	1 portion of fries large size			
2 slices of bacon	two tablespoons of ketchup	1 medium salad with half lettuce 2 tomatoes with 2 tablespoons of olive oil	1 chocolate muffin	
	2 glasses of white wine		1 can of diet coke	
		1 glass of fresh orange juice		

Fill it in on **ONE** of the following days: Monday or Tuesday or Wednesday

Date:

Breakfast	Lunch	Dinner	Snacks	Water

Fill it in on **ONE** of the following days: Thursday, or Friday

Date:

Breakfast	Lunch	Dinner	Snacks	Water

Fill it in on **ONE** of the following days: Saturday or Sunday

Date:

Breakfast	Lunch	Dinner	Snacks	Water

Appendix 5: Published Material

"Cardiovascular" Drugs in Rheumatoid Arthritis: Killing Two Birds with One Stone?

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Abstract: The introduction of statins and drugs blocking the renin angiotensin aldosterone system in the treatment of cardiovascular diseases (CVD) in the general population has led to substantial reductions in morbitity and mortality. Recent evidence suggests multiple actions of these agents, including modulation of the immune response and attenuation of inflammation. Even though several studies have addressed the anti-inflammatory properties of these drugs in the general population, only few studies have focused on their potential benefit when administered to patients with rheumatoid arthritis (RA), a chronic systemic disease characterised by both inflammatory joint damage and excess cardiovascular mortality. The present review focuses on the potential role of these agents in reducing the excess CVD (by controlling cardiovascular risk factors, improving endothelial dysfunction, reducing size and increasing stability of atheromatous plaques, activating the fibrinolytic system and reducing systemic inflammation) and in controlling the disease itself (both systemic and localised joint inflammation), in RA patients. Overall, the review has strong evidence to support the effects of statins on reducing cardiovascular risk, however by comparison the evidence supporting their efficacy in RA is relatively weak.

INTRODUCTION

Diseases of the cardiovascular system are the commonest cause of death in the United Kingdom and consume a significant proportion of the National Health Service Budget [1]. Of the cardiovascular diseases (CVD), coronary heart disease (CHD) is the most prevalent and its magnitude is escalating exponentially. It is predicted that by 2031 the number of cases of CHD will have increased by 44% [2]. Therefore, it is not surprising that the use of drugs such as statins, angiotensin converting enzyme inhibitors (ACE-I), angiotensin II - type 1 receptor blockers (ARBs) and the anti-obesity drugs orlistat/sibutramine/rimonabant are increasingly being used to treat dyslipidaemia, hypertension and obesity respectively, all of which are well-established CHD risk factors.

Patients with rheumatoid arthritis (RA), a chronic inflammatory disease primarily of the synovial joints, have an abnormal lipid profile [3,4], a high prevalence of hypertension [5,6] and a tendency towards a higher body fat:lean mass ratio [7,8]. In RA there may be an increased prevalence of clustering of these risk factors, the metabolic syndrome (MetS) [9,10], which identifies cardiovascular risk beyond the sum of its components and is considered an independent CVD risk factor in its own right [11]. Therefore, therapy with the types of drugs mentioned above may prove of particular cardiovascular benefit in RA patients.

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Although these drugs were initially designed to target specific risk factors for CHD with a net effect of reducing morbidity and mortality, over time it has become apparent that they may have other beneficial actions. In particular, much interest has focused on their immunomodulatory and anti-inflammatory properties [12]. This interest is likely to have been driven by substantial advances in basic science, illuminating the role of inflammatory processes in the pathogenesis of atherosclerosis [13]. It is now thought that inflammation may be the fundamental key element to the development of atherosclerosis, and as a consequence this has spurred the development and adoption of inflammatory biomarkers for cardiovascular risk prediction, such as high sensitivity C-Reactive Protein (hsCRP) [2]. All three classes of drugs (statins, ACE-I/ARBs and anti-obesity medications) have been shown to have anti-inflammatory properties [14-16], thus they may act *via* a two-pronged approach to combat CHD: affect their specific target and modulate the inflammatory response.

Over the last 50 years [17], mounting evidence associates RA with an increased mortality and reduced life expectancy compared to the general population [18,19]. Almost 50% of RA deaths are due to CVD [20] and of these the majority are a consequence of Ischaemic Heart Disease (IHD), particularly myocardial infarction and chronic heart failure. Although conventional cardiovascular risk factors are thought to be more prevalent in RA [4-6, 21, 22], these do not fully account for the increase in ischaemic events, and therefore much of the excess risk has been attributed to novel risk factors, particularly the heightened inflammatory state [23], but also uric acid [24], corticosteroids [25-27] as well as genetic predisposition [28, 29], the exact role of which remains unclear.

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The endothelium is a dynamic structure that plays a pivotal role in cardiovascular homeostasis. Altered endothelial function is now widely accepted to be the earliest stage of atherosclerotic plaque formation [30]. Virtually all known traditional cardiovascular risk factors have been shown to have a negative impact on the endothelium. Interestingly, the majority of these changes can be reversed by effective treatment and correction of the risk factors [31]. In the early stages of the RA disease process, pro-atherogenic changes in endothelial function have been detected in middle-aged patients without CVD risk factors [32]. Endothelial dysfunction in RA has been linked with high inflammatory activity [33], and this notion is supported by an improvement in endothelial function following treatment with disease modifying anti-rheumatic drugs [34]. Therefore, drugs with a dual action (cardiovascular and anti-inflammatory) such as statins or ACE-I/ARBs may prove of particular benefit in RA patients, particularly at the early stages of their disease.

Of note is, that RA patients have less histological evidence of atherosclerosis but greater evidence of inflammation and plaque instability compared to controls at autopsy [35]. It is therefore possible that the scope of benefit in RA patients treated with statins and ACE-I/ARBs *via* the plaque stabilisation actions of these drugs [36-38] may be considerably higher than that observed in states of low grade inflammation. Currently there are no studies to formally assess the effects of statins or ACE-I on atherosclerotic plaque composition or stability in patients with RA.

RA also associates with a hypercoagulable state, represented by elevations of markers of activation of haemostasis, such as fibrinogen, von Willebrand factor (vWF), plasminogen activator inhibitor type 1 (PAI-1) and fibrin D-dimer, and a reduction in fibrinolytic factors such as tissue plasminogen activator (t-PA) [39]. It is possible that this contributes to the increased cardiovascular mortality seen in RA by increasing cellular adhesion to unstable plaques and accelerating thrombus formation following plaque rupture. In the general population, it appears that statins and ACE-I/ARBs can improve the fibrinolytic balance and reduce atherosclerotic plaque thrombogenicity [40, 41]. In RA, in the presence of such an intense prothrombotic environment, it is possible that the anti-thrombotic effects of statins and ACE inhibitors may be even more important; however there are no specific studies to confirm or refute this.

Statins and ACE-I contribute to CVD risk reduction by indirectly intervening at various stages of atherosclerotic plaque development, *via* their effects on common inflammatory pathways. They act to improve endothelial dysfunction, increase nitric oxide (NO) bioavailabilty and antioxidant properties, inhibit inflammatory responses and the activation of the thrombolytic system and stabilise atherosclerotic plaques [42, 43]. (Fig. **1**) Their precise modes of action are discussed in detail later in this review.

Knowledge of the cellular involvement in RA and the mechanisms involved in the disease has improved signifi-

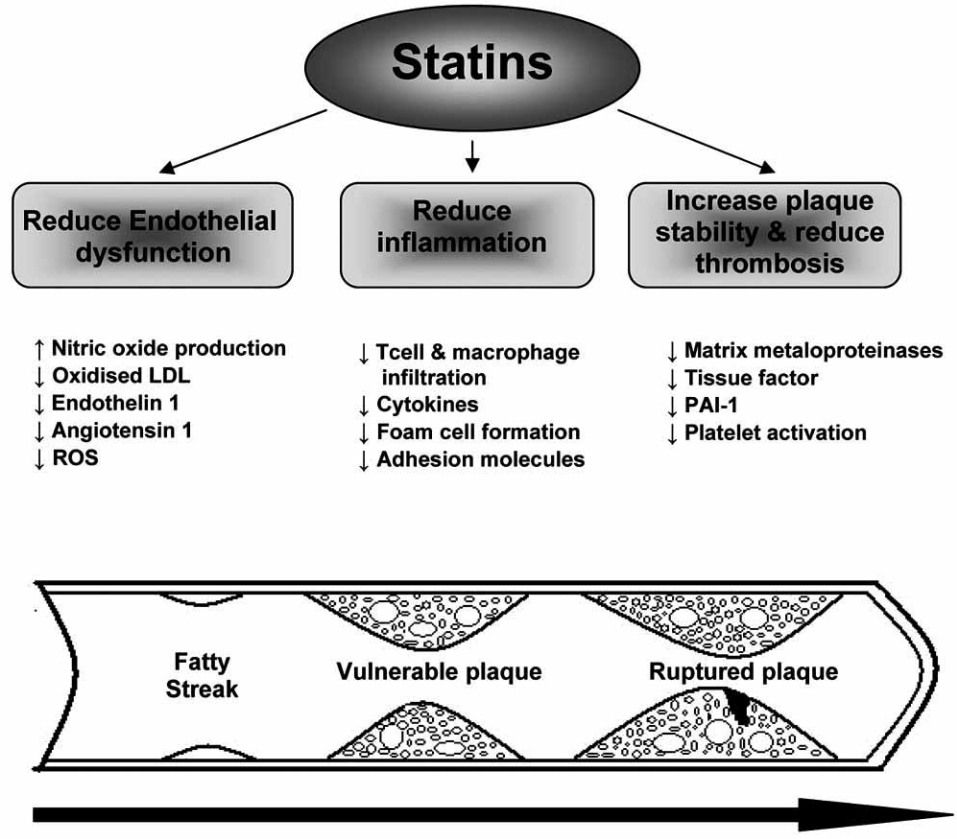

Fig. (1). The pathways by which statins reduce atherosclerotic plaque formation (Adapted from [249]).
LDL: low density lipoprotein, ROS: reactive oxygen species, PAI-1: plasminogen activator inhibitor-1.

cantly. The synovial fluid in active RA is greater in volume and rich in cells, predominantly polymorphs, but also T cells [T helper 1 (Th1) most commonly] and macrophages along with scanty numbers of dendritic cells and B cell derivatives [44]. Nuclear factor-kappa β (NF-κβ), a key transcription factor in the inflammatory cascade, is over expressed in the inflamed synovium [45]. It is a pivotal regulator of the initiation and perpetuation of chronic inflammation [46]. NF-κB regulates the transcription of genes for pro-inflammatory cytokines [eg. tumor necrosis factor-alpha (TNF-α) and interleukin six (IL-6)], adhesion molecules [e.g. intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)], chemokines [e.g. monocyte chemoattractant protein-1 (MCP-1) [47]] and protein subunits of reactive oxygen species (ROS) generating enzymes [e.g. reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase] [48-51]. In RA, NF-κβ activation is inhibited by many conventional anti-inflammatory and disease modifying drugs, such as glucocorticoids and sulphasalazine [52]. Similarly statins [53] and ARBs [54] have demonstrated NFkB inhibitory effects, suggesting a potential disease modifying role of the latter.

Many striking parallels have been drawn between the synovium of an actively inflamed joint in RA and the atherosclerotic plaque [55]. These include the infiltration by similar populations of pro-inflammatory cells (mainly macrophages and T cells) [56], collagen degradation, local expression of adhesion molecules (ICAM-1, VCAM-1 and E-selectin) [57, 58] and neoangiogenesis [59, 60], suggesting that largely similar inflammatory pathways are involved in the pathogenesis of RA synovitis and atherosclerotic CHD [61]. Thus, the pleiotropic immunomodulatory, anti-inflammatory effects of statins, ACE-I/ARBs and anti-obesity drugs, sparked speculation over their potential benefit in RA, both as therapy able to reduce the increased cardiovascular risk, as well as modify joint disease, thus "killing two birds with one stone".

In this review, after providing some pharmacological information about each of these classes of drugs, we discuss in more detail the mechanisms by which they reduce cardiovascular risk and may modify the course of RA.

Statins

Pharmacology

The HMG CoA reductase inhibitors, statins, first introduced in the 1980's, have revolutionised the treatment of hypercholestrolaemia, and are a most important component of primary and secondary prevention of CHD [62].

Statins are competitive inhibitors of HMG-CoA reductase, the rate-limiting step in cholesterol biosynthesis. The binding of a statin to the HMG-CoA reductase active site triggers an alteration in the enzyme, preventing HMG-CoA reductase from attaining a functional structure. The change in conformation at the active site makes these drugs very effective and specific. Through this, they prevent the conversion of HMG-CoA to mevalonic acid, resulting in decreased hepatic cholesterol synthesis, which in turn promotes upregulation of LDL receptors and subsequent removal of LDL from plasma [63]. However, by inhibiting mevalonic acid synthesis, statins also prevent the synthesis of other intermediates downstream, such as farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP). These substrates are crucial for multiple cellular functions and may also have immuno-modulatory potential *via* their interaction with other signalling pathways. An example is the indirect effect of GGPP modulation by statins resulting in activation of peroxisome proliferator-activated receptors (PPAR) α [64], and subsequent inhibition of inflammatory pathways. It is through such mechanisms that statins may exhibit their pleiotropic, anti-inflammatory effects [65] (Fig. **2**).

Statins are classified according to: the pathway used when metabolised by the liver; the process by which they are obtained; their physico-chemical properties; and their active forms. The metabolism of statins in the liver is largely either *via* the cytocrome P450, CYP 3A4 pathway as for simvastatin, atorvastatin and lovastatin, or *via* the CYP 2C9 pathway as used by Fluvastatin and Rosuvastatin [66]. Pravastatin has only minimal interaction with the cytocrome P450 system and much of its metabolism is dependant on glucuronidation [67]. This may be important in terms of their potential for drug interactions, particularly in conditions characterised by polypharmacy [68], such as RA [26]. Statins can be obtained either by fungal fermentation (pravastatin, lovastatin, simvastatin), or by synthesis (fluvastatin and atorvastatin). The division of statins according to their physico-chemical properties allows distinction between those that are hydrophilic (pravastatin), hydrophobic (simvastatin, lovastatin, atorvastatin), and those that display intermediate characteristics (fluvastatin) [69]. The final classification system, distinguishes between statins that are metabolically active on administration (atorvastatin, fluvastatin, pravastatin) and those that are administered as inactive compounds requiring enzymatic modification to the active form (simvastatin, lovastatin) [66].

Cardiovascular Risk Reduction

Control of Risk Factors

Statins are primarily used for the reduction of total cholesterol (TC) and low-density lipoproteins (LDL), however, they have also been found to affect other aspects of the lipid profile, such as high density lipoproteins (HDL) and triglycerides (TG). Total cholesterol is reduced by 15-40%, LDL by 20-60% and TG by 10-30%, whereas HDL can increase by 5-15% [70]. The relative improvement observed in the lipid profile varies between the different drugs and can be explained through the differences in chemical structure and route of metabolism. Rosuvastatin appears to be more potent than atorvastatin, simvastatin, or pravastatin at equivalent doses, resulting in a significantly greater reduction in LDL levels [71]. It is hypothesized that the differences in potency may also influence their ability to exhibit their anti-inflammatory properties, but this remains to be proven.

The landmark Scandinavian Simvastatin Survival Study trial (4S) established the benefits of statins on mortality in patients with atherosclerosis [62]. A meta-analysis of 34 trials, studying the association of statin use and CHD in secondary prevention, has confirmed a 13% overall reduction in CV mortality [72]. Subsequent, large multi-centre, randomised controlled trials (RCTs), including the West Of Scotland

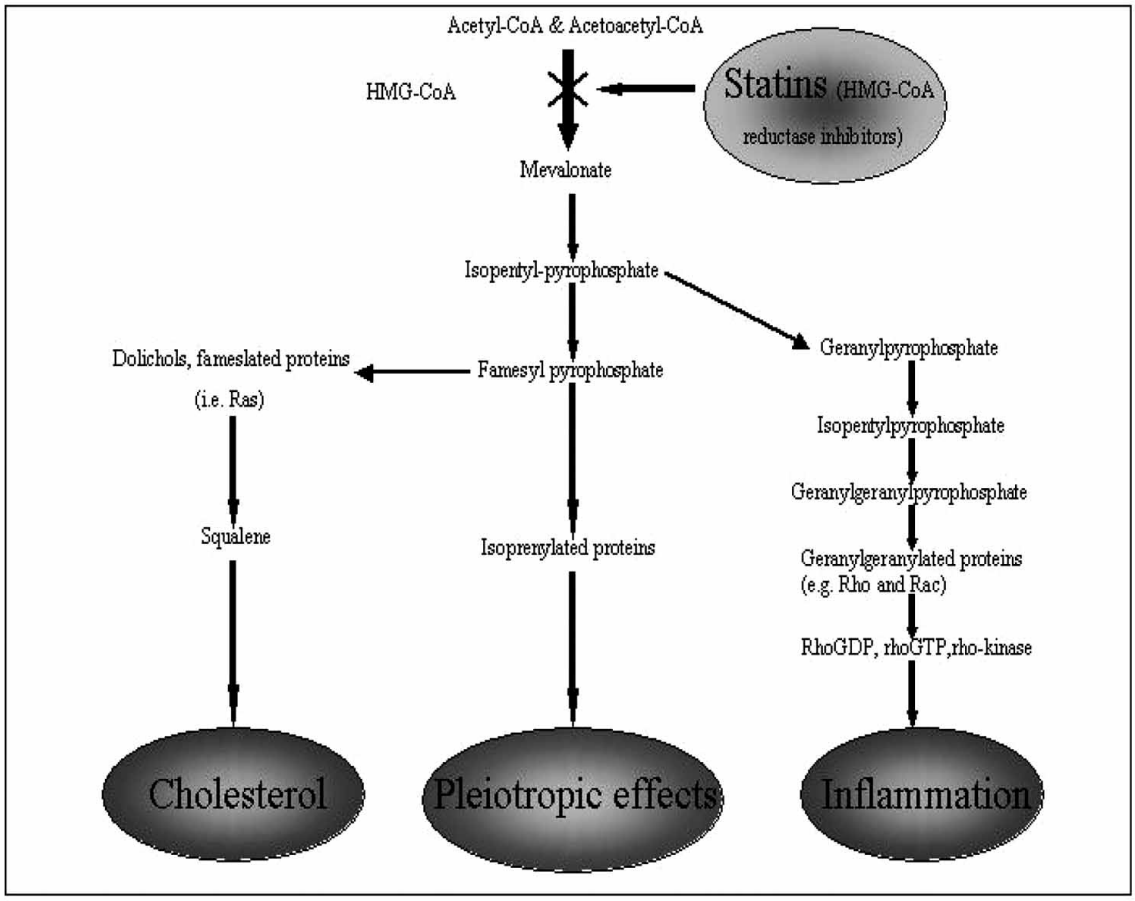

Fig. (2). Mechanism of action of statins.

HMG CoA: 3-hydroxy-3-methyl-glutaryl-CoA reductase, Ras: a small G protein, Rho: A member of the Ras superfamily, RhoGDP: inactive form of Rho Protein, RhoGTP: active form of Rho Protein, Rho-kinase: a serine/threonine-specific protein which is activated by GTP-bound Rho.

Coronary Prevention Study (WOSCOPS) [14], the Anglo-Scandinavian Cardiac Outcomes Trial –Lipid Lowering Arm (ASCOT-LLA) [73], and the air force/Texas coronary atherosclerosis prevention study (AFCAPS/TexCAPS) [74], have reinforced the 4S findings, as well as affirming a reduction in cardiovascular (CV) morbidity and mortality in patients without established atherosclerosis. These findings were irrespective of age, gender or baseline cholesterol concentration.

Some of the vascular benefits arising from statin treatment may associate with blood pressure control and arise from their inhibitory effects on NF-kβ. Statins have been shown to reduce NF-kβ activation in vascular smooth muscle cells and mononuclear cells [53], and abolish TNF-alpha-induced NF-kβ activation in human endothelial cells [75]. Furthermore, statins provide some of their anti-oxidant properties that preserve endothelial function by attenuating angiotensin II-induced free radical production in the vascular smooth muscle cells by inhibiting NAD(P)H oxidase activity and down-regulating AT 1 receptor expression [76]. These actions may be particularly pertinent to states of high-grade inflammation, such as RA. Arterial stiffness was found to be significantly reduced in 29 patients with RA treated with atorvastatin [77], and these changes were more pronounced in patients with higher levels of inflammation. Collectively, the mechanisms outlined above may act upon central or peripheral control of blood pressure to contribute to the reported anti-hypertensive effects of statins [78, 79]. In 2007, a meta-analysis of 20 randomised control trials, including 828 hypercholestrolaemic patients on stable anti-hypertensive medication, has confirmed a small but statistically significant effect of statins on blood pressure in the general population [80], however, there have not been any studies to endorse these findings in patients with RA.

Improvement of Endothelial Function and Anti-Oxidant Potential

Statins preserve endothelial function by inhibiting the expression of adhesion molecules, such as VCAM and ICAM, thus reducing leukocyte recruitment and inflammation [81]. Changes in endothelial function and endothelium dependant vasomotion have been observed as early as 24 hours after initiation of statin treatment, in normocholestrolaemic and hypercholestrolaemic patients [82, 83]. Statins have a positive impact on nitric oxide bioavailability, by up-regulating endothelial nitric oxide synthase (eNOS) expression [84, 85], and reversing the inhibitory effect of oxidised

LDL on eNOS [86,87]. The overall impact is improved NO-dependant vasorelaxation. A single study specifically in RA showed that patients treated with simvastatin 40mg, had improved endothelial function as measured by flow mediated dilation, and this was greater in those with higher baseline CRP levels; there was also a decrease in oxidised LDL [88].

Statins do not only reduce circulating oxidised LDL but they also inhibit their uptake by macrophages [89], and thus subsequent foam cell formation, a critical step in atherosclerosis. Patients treated with atorvastatin may have reduced macrophage uptake of oxidised LDL as a consequence of decreased activity of macrophage CD36, a recognised receptor for oxidised LDL [90]. However, this mechanism is now under scrutiny as a recent study has shown conflicting results, indicating upregulation of CD36 on macrophages with statin therapy, and an additive effect of PPAR-γ ligands to this phenomenon [91].

Reduction of Plaque Size and Increase in Plaque Stability

Although all stages of plaque formation are important and should be considered as potential targets for therapy, it is plaque instability and rupture that have the most devastating clinical consequences, as they are responsible for the acute coronary syndromes (ACS). Statins contribute to plaque stability by reducing plaque size or by altering physicochemical properties [36]. The infiltration of atherosclerotic plaques by monocytes/macrophages is reduced, and secretion of proteolytic enzymes, matrix metaloproteinases (MMPs) that contribute to thinning of the fibrous atherosclerotic cap is decreased in patients receiving statin therapy [92] (Fig. **1**). In one study, patients received either pravastatin 40mg or placebo for 3 months prior to carotid endarterectomy. The plaques in those treated with pravastatin had a more stable morphology with a lower lipid content, less oxidised LDL, fewer macrophages and T cells, less MMP-2, more tissue inhibitor of MMP-1 and a higher collagen content [93].

In two recent trials, REVERSAL [94] and ASTEROID [95], statins have been shown to induce coronary plaque regression. REVERSAL compared the effects of atorvastatin and pravastatin on coronary plaque progression by intravascular ultrasound. In patients receiving atorvastatin, a reduction in the progression of coronary atherosclerosis was observed, however, these results were not mirrored in the pravastatin arm where atherosclerotic lesions progressed. ASTEROID produced impressive results, with a significant reduction in plaque volume following treatment with rosuvastatin over a two-year period. The observed plaque regression was linked to a significant reduction in their LDL levels (53.2%) and an increase in their HDL levels (14.3%). Although these results are promising, the trial was limited by the lack of a control group.

Patients with RA have a worse outcome and a higher re-infarction rate after an ACS [96], and although direct evidence is lacking, this has been attributed at least in part to increased instability of atherosclerotic plaques associated with the enhanced inflammatory environment and the presence of CD4+CD28- T cells [97]. Plaque stabilisation through statin (or other) therapies may therefore be particularly important in RA, but there are no studies addressing this in the RA population.

Any studies performed within an RA population should take into account the possibility that the inflammatory burden may actually alter the functional capacity of these drugs, and as a consequence higher doses may be required to gain clinical benefit. A recent study suggests for example that inflammatory cytokines disrupt LDL-receptor feedback regulation and cause a degree of statin resistance [98].

Effects on the Fibrinolytic System

Statins exert their positive effects on the fibrinolytic system by lowering levels of PAI-1 and increasing levels of t-PA in hypercholestrolaemic patients. However, such results have not been replicated in patients with mixed-type hyperlipidaemia [99]. A study performed on rats demonstrated a 3-fold increase in tPA activity and a reduction in PAI-I activity, in those treated with a statin [100]. A reduction in plasma fibrinogen levels and blood viscosity has also been observed in type 2 hypercholestrolaemic patients treated with pravastatin but not with simvastatin [101]. Several investigators have also reported additional effects of statins on platelet aggregation, with a reduction in platelet deposition on damaged vessel walls at high shear stress rates observed with both atorvastatin and pravastatin [102-104]. These findings may be attributed to a reduction in platelet membrane RhoA expression [103] and plaque levels of tissue factor [105]. There are no studies of the effects of statins on the fibrinolytic system in patients with RA.

Reduction of Systemic Inflammation

The hypothesis that statins provide benefit beyond their lipid lowering effects is supported by evidence provided in multiple large clinical trials, including CARE (Cholesterol And Recurrent Events) [106] and WOSCOPS [14]. They indicate that despite comparable serum cholesterol levels amongst the statin and placebo groups, statin-treated individuals had a significantly lower risk of CHD than age-matched placebo controls [106-108]. The CARE trial of pravastatin vs placebo demonstrated an improvement in baseline CRP levels and a marked risk reduction in those with elevated baseline CRP, despite comparable LDL levels [106]. The sub-analysis of the Pravastatin or Atorvastatin Evaluation and Infection – Thrombolysis in Myocardial Infarction (PROVE IT-TIMI) 22 trial, suggests that the clinical benefits achieved with statin therapy in patients with ACS are greater in those with a lower CRP level post statin therapy than those with higher CRP levels, irrespective of the resultant level of LDL cholesterol [109]. These trials indicate that the benefit of statin therapy may extend to patients with a low total and LDL cholesterol, but with a heightened inflammatory state as measured by their CRP (10), such as those with RA. In the general population, this hypothesis is currently being evaluated by the JUPITER (Justification for the Use of statins in Primary prevention: an Intervention Trial Evaluating Rosuvastatin) trial [110], where 17,802 healthy individuals with low LDL cholesterol (<130) but elevated high sensitivity CRP (>/=2) are being randomised to either Rosuvastatin or placebo. A large RCT in RA is also underway, the TRial of Atorvastatin for the primary prevention of Cardiovascular Events in patients with Rheumatoid Arthritis (TRACE RA) [111] : this is the first and largest hard end-point trial of statin therapy in patients with RA in the world, and much needed, as this population has been

systematically excluded from all large statin trials to date. This pivotal trial, will hopefully provide definitive evidence about the role of statins in the primary prevention of cardiovascular events in RA, but will also produce useful information about effects of statins on disease activity, biochemical and metabolic parameters, blood pressure and the prevalence of side effects.

RA Disease Modifying Potential

Reduction of Systemic Inflammation

The anti-inflammatory actions of statins in RA have been formally assessed by a double-blind randomised placebo-controlled Trial of Atorvastatin in RA (TARA), involving 116 RA patients [112]. Participants were allocated either to atorvastatin 40mg or placebo, in addition to their existing disease modifying therapy (DMARD). Despite randomisation, the atorvastatin arm included more patients taking methotrexate and there was some usage of corticosteroids in both arms during the course of the trial. Notwithstanding these shortcomings, at six months, there was a significantly higher fall in disease activity score (DAS28), swollen joint count, CRP and ESR in the atorvastatin arm compared to placebo, providing promise that statins may mediate clinically apparent anti-inflammatory effects both on haematological and clinical markers of disease activity in RA. The investigators of a subsequent large observational study involving 4152 RA patients, described similar results with a significant reduction in CRP levels, but failed to demonstrate a change in DAS 28 or the Health Assessment Questionnaire (HAQ), a proxy of disease severity [113].

Effects on RA Synovitis

In RA, statins may also act at a more local level, on the synovium. This mode of action can be attributed both to their anti-inflammatory properties and their effect on the lipid content within the synovial fluid [114, 115].

There is good evidence that statins act by inhibiting the production of cytokines such as IL-6 and IL-8 [116, 117], as well as by reducing the expression of MCP-1 mRNA in the joint with an overall effect of reducing the number of inflammatory cells found in the synovial membrane [118]. In a murine model of collagen-induced arthritis, simvastatin was shown to have anti-arthritic effects by suppression of the Th1 immune response [119]. Significant immuno-modulatory actions of low dose simvastatin on the Th1 pathway have been observed in a recent study of 28 patients with RA [120]. At 12 weeks, immunological assessment revealed a reduction in the Th1/Th2 and CD4/CD8 ratios, along with a significant reduction in median levels of biochemical markers of inflammation (CRP, ESR and Rheumatoid factor). One report suggests that lipophilic statins, such as fluvastatin, exert novel anti-inflammatory effects by inducing synovial cell apoptosis in RA [121]; hydrophilic statins do not appear to have a similar action, indicating this is unlikely to be a class effect. Another report suggests that statins attenuate the insertion of GGPP into the plasma membrane with a resultant decrease in viability and an increased apoptosis of synovial fibroblasts [122].

As far back as the 1960's, major differences were observed between the lipid content of synovial fluid obtained from inflamed joints of RA patients, compared to fluid from normal joints [114]. In RA, the level of all lipid components in the synovial fluid is increased significantly, with the most dramatic elevations occurring in cholesterol and phospholipids [114]. It is known that the synovial fluid lipids and phospholipase A2 activity correlate well with RA disease activity [123, 124]. This phenomenon may be partially due to the high levels of IL-1 found in RA, stimulating synoviocytes to release phospholipase A2 into the extracellular space [125]. There is now evidence demonstrating that an increase in synovial fluid lipoproteins may contribute to the synovitis in RA through participation in the arachidonic acid pathway within the joint space [126]. Statins may have the ability to alleviate much of this by modifying the lipid profile, particularly cholesterol levels within the joint. Yet again, there is no direct evidence from clinical trials of statins in RA to support or refute this hypothesis.

ACE-I AND ARBs

Pharmacology

ACE-I were developed in the late 60s from the venom of the Brazilian pit viper, Bothrops jararaca [127]. Over thousands of years, the same environmental pressures that forced the Yanomamo Indians [128] and the terrestrial animals in northern Brazil to evolve a hyperactive renin-angiotensin-aldosterone system (RAAS), also led Bothrops jararaca to conserve an efficient killing mechanism that targeted its enemies' haemodynamic vulnerabilities. Modern hypertension may represent the kidney's attempt to reconcile a mismatch between conserved RAAS activity and high dietary salt intake. This results to pressure natriuresis, which helps establish the salt homeostasis at the expense of hypertension-related cardiovascular and renal damage [129].

After numerous animal and clinical studies, the first ACE-I to be approved by the USA Food and Drug Administration (FDA) was captopril in the early 80's [127]. ACE-I are classified in three categories according to the group that binds the zinc atom of the ACE molecule: those containing a sulfhydril (benazepril, captopril, zofenopril), a carboxyl (cilazapril, enalapril, lisinopril, perindopril, quinapril, ramipril, spirapril, trandolapril) or a phosphoryl (fosinopril) group as zinc ligand [130]. ACE-I competitively inhibit the angiotensin converting enzyme, which is involved in the metabolism of many small peptides including the conversion of angiotensin I, an inactive octapeptide, into angiotensin II. Kininase, an enzyme that catalyses the degradation of bradykinin and other potent vasodilator peptides, is also competitively inhibited by ACE-I, leading to increases of bradykinin levels, which in turn stimulate the B2 receptors leading to the release of nitric oxide (NO) and vasoactive prostaglandins (prostacyclin and prostaglandin E2) [131, 132].

There are a number of approved nonpeptide selective blockers of the binding of angiotensin II to type 1 (AT1) angiotensin receptors on the cell membrane, thereby inhibiting the action of angiotensin II [133]. The first ARB, losartan, was licensed for use in clinical medicine in the treatment of hypertension in 1994 [134]. Subsequently, five other members of this class of compound have been licensed in the United Kindom, valsartan, irbesartan, candesartan, telmisartan and eprosartan [134].

Recently the cloning of an ACE homologue [135, 136] with different substrate affinities and insensitivity to blockade with ACE inhibitors prompted a reconsideration of the accepted thinking about the biochemical constituents, mode of action, and function of RAAS. The complexity of the integration responsible for the formation and degradation of molecules participating in vascular tone, growth and cellular signalling is demonstrated in Fig. (**3**).

The system not only shows a high degree of hierarchy, but it also displays modularity because functionally linked molecules (or nodes) are working together to achieve a relatively distinct function. These important concepts underscore the weakness of the previously accepted view of the system as a linear and sequential biochemical cascade primarily responsible for the formation of angiotensin II [137]. Visualization of the interplay between the two ACEs in determining product formation and metabolism may be particularly useful in interpreting the effects of ACE-I because blockade of the ACE enzyme will not only suppress the formation of angiotensin II but also inhibit the metabolism of both angiotensin (1-7) and bradykinin, leading to increased levels of the latter two (Fig. **3**). Similarly, apart from antagonizing angiotensin II action on AT1 receptors, ARBs lead to angiotensin (1-7) level augmentation due to facilitation of the conversion of angiotensin II to angiotensin (1-7) and due to a renin-mediated increase in angiotensin I (reflecting the blockade of the negative feedback that AT1 receptors exert on renin release) (Fig. **3**) [138]. The anti-inflammatory properties of ACE-I and ARBs are thought to be due to inhibition of the action of angiotensin II on AT1 receptors, which enhances inflammatory responses *via* several mechanisms, and due to increased levels of angiotensin (1-7), which may act as an anti-inflammatory agent [138]. ARBs may offer an additional advantage by activating the AT2 receptor, which promotes anti-proliferation, differentiation and vasodilation [139, 140]. The significant role of angiotensin II-independent

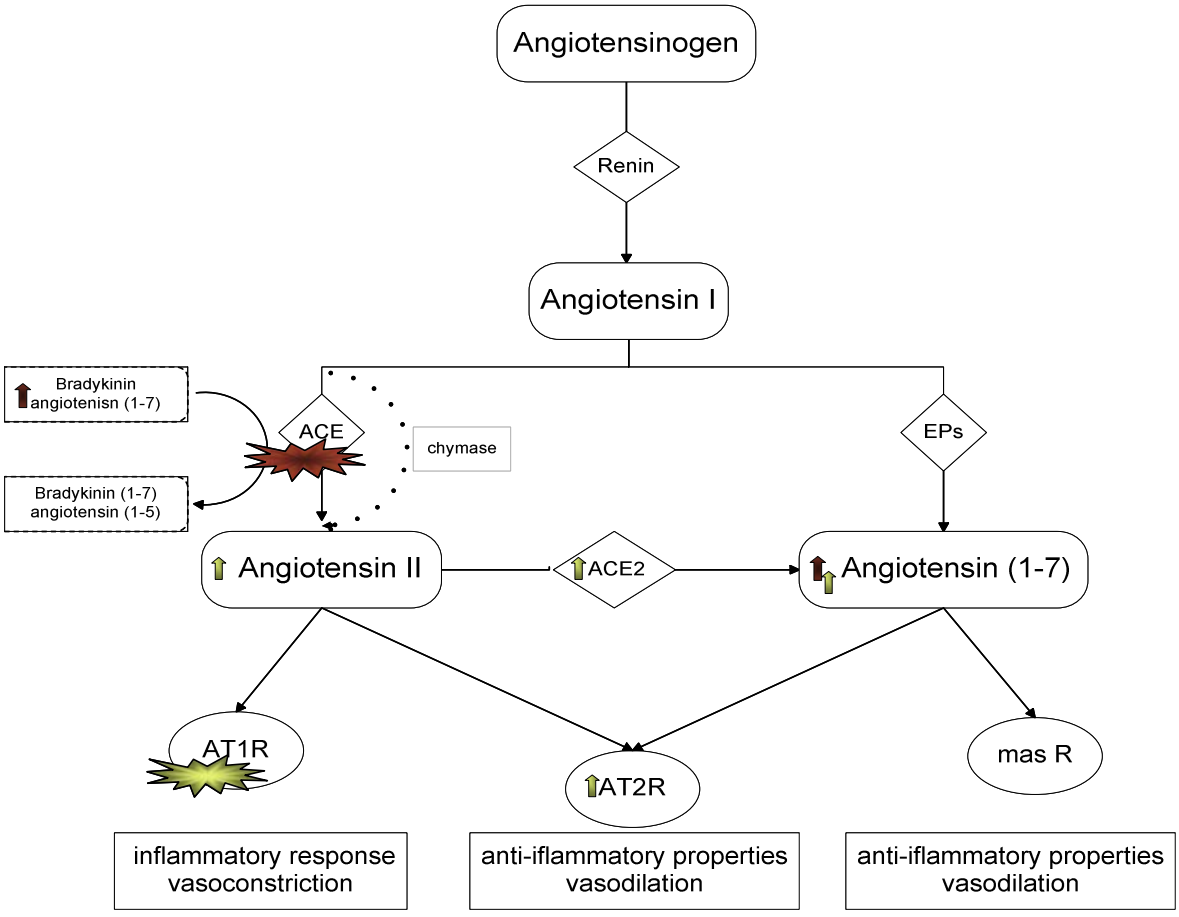

Fig. (3). The complexity of the renin angiotensin aldosterone system and the nodes were ace inhibitors and angiotensin II receptor type 1 blockers exert their effects.

Due to blockade of the ACE enzyme (red star), ACE-I will not only suppress the formation of angiotensin II but also inhibit the metabolism of both angiotensin (1-7) and bradykinin, leading to increased levels of the latter two (red up-arrows). Similarly, apart from antagonizing angiotensin II action on AT1 receptors (yellow star), ARBs lead to angiotensin (1-7) level augmentation due to facilitation of the conversion of angiotensin II to angiotensin (1-7) (yellow up-arrows).

LDL: low density lipoprotein, ROS: reactive oxygen species, ACE: angiotensin converting enzyme, AT: angiotensin, EP: endopeptidases, mas R: mas receptor, ACE-I: ace inhibitors, ARBs: aniotensin receptor blockers.

mechanisms, which probably reflect increased levels of angiotensin (1-7), in the anti-atherogenic and anti-inflammatory effects of ACE-I has been demonstrated on apolipoprotein E-deficient mice, which were co-administered angiotensin II and enalapril [141].

Cardiovascular Risk Reduction

Control of Risk Factors

Hypertension is one of the major classical risk factors for CVD and accounts for approximately 18% of the general population attributable risk of a first MI [142]. Several studies among patients with RA have demonstrated that it associates with subclinical atherosclerosis [143-145] and is one of the most significant independent predictors of CVD, with relative risk ranking from 1.49-4.3 [146-148]. Using data from the Framingham heart study in the US and the Third (US) National Health and Nutrition Examination Survey (NHANES III), it was projected that a 20 mmHg increase in systolic BP in RA patients would associate with 1572 additional ischaemic heart disease events and 602 additional stroke events over 1 year [149]. ACE-I and ARBs are first line treatment in most cases of hypertension [150,151], with 50-60% of Caucasian patients demonstrating a good response to monotherapy [152, 153]. In the CONSENSUS trial [154], patients in NYHA class IV heart failure (HF) were followed for an average of 188 days. Mortality at 6 months was significantly reduced in the ACE-I group (enalapril) (26% vs. 44% in the control group). In SOLVD [155], patients in NYHA class II and III HF were followed for a mean of 3.45 years. The cumulative mortality was 39.7% in the placebo group compared to 35.2% in the active treatment group. In the meta-analysis of the ACE-I in Myocardial Infarction Collaborative Group, including over 100,000 patients [156], mortality at 30 days was reduced from 7.6% in the placebo group to 7.1% in the ACE-I group. Meta-analysis of late intervention (treatment initiated >48h after MI and continued long term) trials [157], mortality was reduced from 29.1% to 23.4% with ACE-I therapy after an average follow-up of 2.6 years. Later on, randomised controlled trials comparing ACE-Is with ARBs demonstrated similar effects of these agents on the reduction of mortality in patients with Heart Failure (HF) or MI [158, 159]. In a recent meta-analysis of 38.080 chronic heart failure patients [160], ARBs were associated with reduced all-cause mortality (OR=0.83, 95%CI, 0.69 to 1.00) and HF hospitalizations (OR=0.64, 95%CI, 0.9 to 1.26) compared to placebo. The above beneficial effect of ACE-I/ARBs on CVD mortality is partly explained by their anti-proliferative effects (reduction of vascular and cardiac hypertrophy and extracellular matrix proliferation) and reduction of ventricular remodelling after myocardial infarction [161, 162].

In addition, ACE-I/ARBs are used for risk reduction in particular populations at risk, due to beneficial renal effects: they prevent progression of microalbuminuria to overt proteinuria [163], attenuate the progression of renal insufficiency in patients with a variety of non-diabetic nephropathies [164] and prevent or delay the progression of nephropathy in patients with insulin-dependent diabetes mellitus [165].

A pertinent question for patients with high-grade inflammation, such as those with RA, is whether inflammation affects in any way the potency of these pharmacological agents. At least for ARBs, inflammation appears to have no down-regulating effect, therefore not altering the anti-hypertensive effect of drugs such as valsartan [166] and losartan [167] in RA patients. On the contrary, a trend towards up-regulation of the above receptors is evident, which favours treatment with ARBs in this patient group. Interestingly, even though there are reduced concentrations of losartan's active metabolite EXP 3174 (because of inhibition of the metabolising enzymes CYP2C9 and CYP3A4) amongst RA patients compared to controls, the potency of AT1R antagonists does not appear to be reduced by inflammation [168]. The latter could be attributed possibly to a pharmacological effect of the parent drug and/or AT1R upregulation of inflammatory mediators.

Improvement of Endothelial Function And Anti-Oxidant Potential

Angiotensin II has been shown to play a role in neointimal monocyte infiltration and endothelial dysfunction through NF-kβ activation and MCP-1 expression in a model of accelerated atherosclerosis in rabbits [169]. It is thought therefore, that inhibition of angiotensin II may reduce endothelial infiltration by monocytes, restore endothelial function and ultimately inhibit progression of atherosclerosis.

Accumulating evidence suggests that oxidant stress (mediated by ROS) alters many functions of the endothelium, including modulation of vasomotor tone [170]. Many ROS possess unpaired electrons and thus are free radicals. These include molecules such as superoxide anion ($O_2^{\cdot-}$), hydroxyl racial ($HO^{\cdot}$), nitric oxide ($NO^{\cdot}$), and lipid radicals. NADPH appears to be the major source of superoxide in blood vessels [171]. Incubation of human umbilical vein endothelial cells (HUVECs) with angiotensin II has been shown to result in a time- and dose-dependent induction of superoxide $O_2^{\cdot-}$ formation, which was associated to similar dose-dependent regulation of NADPH oxidase subunit gp91-phox [15]. In the same study, AT1 receptor blockade therapy, but not ACE-I therapy, before coronary bypass surgery, was shown to down-regulate gp91-phox expression in internal mammary artery biopsies of patients with coronary artery disease (CAD). In a cross-sectional study of 33 normotensive patients with stable CAD, superoxide level decreased by 52% after a 24-week period of irbesartan treatment [172]. In another RCT, candesartan significantly reduced plasma levels of malondialdehyde, the end-product of lipid peroxidation by ROS [173]. Similarly another RCT demonstrated significant reductions in plasma levels of 8-isoprostane, a marker of oxidative stress, in the irbesartan group compared to placebo [174]. This reduction in ROS generation would also potentially reduce oxidative damage to lipids, amino acids and proteins and therefore lead to lower levels of oxidized LDL, which is crucial in the formation of foam cells [175]. Indeed in a study of 47 patients with documented CAD who were treated for 12 weeks with irbesartan, lipid peroxidation decreased by 36% in comparison to placebo [176].

The ability of ACE-I and ARBs to prevent or restore endothelial function [177] may also be explained, in part, by a shift of the balance between vasoconstrictive forces (angiotensin II) and vasodilatative factors (bradykinin and NO) towards vasodilatation. Evidence supporting this notion has

been provided by a randomized, double-blind, placebo-controlled, crossover study comparing placebo and candesartan 16 mg daily over two months in 45 patients with mild-to-moderate hypertension [173]. Compared to placebo, candesartan improved significantly the percent flow-mediated dilator response to hyperaemia. Similar results were obtained in a randomised double blind control trial of irbesartan vs. placebo in patients with the Metabolic Syndrome (MetS). After 4 weeks of therapy, endothelium dependent flow mediated vasodilation of the brachial artery was increased by 67% in the irbesartan group compared to placebo [174].

In addition to these findings, there has been speculation over the effect of ACE-I on the rate of endothelial cell apoptosis. The current theory is that ACE-I may correct endothelial dysfunction as a consequence of a reduction in the rate of endothelial cell apoptosis. A recent study [178], confirmed inhibition of endothelial cell apoptosis *in vivo* following administration of ACE-I, which was most marked with perindopril; interestingly these results were not replicated in the *in vitro* arm of the trial carried out on HUVECs [178], which reinforces the usual conundrum of how transferable *in vitro* observations are in the *in vivo* situation, and vice versa.

Despite the increased prevalence of endothelial dysfunction in RA patients, and the compelling evidence regarding the beneficial effect of ACE-I/ARBs on endothelial function, to date there is only sparse data examining the effect of these drugs on endothelial function in RA. A study involving 45 RA patients who were randomised either to placebo, simvastatin (20mg/day) or quinapril (10mg/day), demonstrated a significant lowering of CRP and TNF-a and improvement in endothelial function in the simvastatin arm, along with a tendency to an increase in endothelium-dependent vasodilation in those treated with quinapril. It is possible that the limited efficacy of quinapril may be related to inappropriate dosage or inadequate length of treatment [179]. Further larger scale trials are required in RA to evaluate the effects of ACE-I on endothelial function.

Reduction of Plaque Size and Increase in Plaque Stability

Angiotensin II, AT1 receptors and ACE are expressed at strategically relevant sites of human coronary atherosclerotic plaques in the shoulder region and may contribute to inflammatory processes within the atherosclerotic vascular wall and to development of acute coronary syndromes [180]. In apolipoprotein E-deficient mice, 4 weeks of subcutaneous admistration of angiotensin II induced histomorphologic features of unstable plaque (increased foam cell area, intralesional neovasculature and haemorrhage, active matrix MMP-2 colocalization within macrophage foam cells and increased MCP-1 and VCAM expression) [181]. The effects of angiotensin II antagonism on extracellular matrix components of advanced atherosclerosis have been assessed in twenty four week old apolipoprotein E-deficient mice [182]: although there was no difference in the macrophage component, angiotensin II antagonism increased the relative collagen portion of the lesions, lessened elastin fragmentation, increased the total elastin content of the aorta and reduced the mRNA and activity/protein of the elastolytic proteases, cathepsin S and MMP-9. In line with these are the results of another study [38] which demonstrated an attenuation of the degree of atherosclerosis, increased collagen production and lower percentage of macrophage to total plaque amongst candesartan-treated rabbits. These effects are thought not only to decrease further expansion of advanced lesions but also to stabilize the established atherosclerotic plaques and may underlie the decreased incidence of acute cardiovascular events that are observed in patients in whom angiotensin II antagonism is begun after atherosclerosis is already established. In a small non-randomised study [37], administration of an ARB associated with a reduction in coronary plaque burden as assessed by intravascular ultrasound, which implies a more favourable long term outcome in patients with coronary atherosclerosis. So far, no relevant data exist in patients with RA.

Effects on the Fibrinolytic System

ACE-I also modulate vascular fibrinolytic balance by decreasing angiotensin II, a potent stimulus for PAI-1 synthesis and by increasing bradykinin levels, a potent stimulus for tissue plasminogen activator [183]. Thus, ACE-I lowers plasminogen activator inhibitor type 1 (PAI-1) concentrations and the molar ratio of PAI-1/t-PA. ACE-I also counteract the platelet aggregation induced by angiotensin II since they increase the production of NO and prostacyclin. In a study of type 2 DM patients with a diagnosis of mild essential hypertension, 129 patients were randomly allocated to candesartan or enalapril treatment. Patients in both treatment groups had significant reductions in their serum levels of vWF, fibrinogen and PAI-1 [184]. Again, there are no studies addressing these potential effects in patients with RA.

Reduction of Systemic Inflammation

In several clinical trials, ARBs and ACE-I demonstrate an anti-inflammatory effect by lowering the levels of inflammatory mediators such as IL-6, TNF-α and TNF-a receptor [15, 172, 174, 184, 185], adhesion molecules (ICAM-1, VCAM-1) [172, 184-186], chemokines (monocyte chemo attractant protein MCP-1) [173] and acute phase proteins such as C-reactive protein [185, 187, 188] and serum amyloid A [189]. These effects seem to be independent of the degree of blood pressure reduction [188].

The first *in vivo* study regarding the effect of either ARBs or ACE-I on either reactive oxygen species (ROS) generation or other mediators of inflammation came from Dandona *et al.* [190] who studied four groups of 8 subjects who were given valsartan, quinapril, simvastatin or placebo for one week. Their data showed that valsartan inhibited ROS generation by both polymorphonuclear cells (PMN) and mononuclear cells (MNC) and suppressed NF-kB and its pro-inflammatory properties. The ineffectiveness of quinapril to suppress inflammatory mediators was mainly attributed to the slower reduction of angiotensin II levels in patients administered ACE-I. CRP fell significantly over 30% in the first week, a magnitude that is greater than the one reported in a different study (14%) following pravastatin therapy for 6 months [191].

Of note is, that the anti-inflammatory effects of Valsartan (i.e. reduced expression of MCP-1, TNF-a, IL-6 and IL1b alongside reduced infiltration of leukocytes and macrophages in the injured arteries) were attenuated in AT2 receptor knock out mice, therefore suggesting that the stimulation of AT2 receptor after AT1 blockade is important in the im-

provement of the inflammatory vascular injury [192]. Telmisartan, an AT1 receptor antagonist, acts upon T cells to inhibit the expression of a pro-inflammatory integrin (beta2-integrin MAC-1). These effects are independent of angiotensin II and therefore suggest an AT1 receptor independent atheroprotective effect of Telmisartan [193].

RA Disease Modifying Potential

Reduction of Systemic Inflammation

ACE-I and ARBs express some of their anti-inflammatory properties by modulating the balance of T cell subsets (Th1-Th2). A study performed on rats, demonstrated an imbalance towards a Th1 phenotype and an associated elevation in IFN-γ and IL-4, when infused with angiotensin II. Subsequent treatment of the rats with candesartan corrected this imbalance [194]. It is through the action of these drugs on T cells that clinical benefit in predominantly Th1-driven diseases, such as RA, may be observed, but this remains to be proven. The first clinical trial exploring the use of ACE-I as a potential treatment for RA took place in the mid 80s, when no improvement was demonstrated in RA clinical features or ESR in three RA patients after 16 weeks of treatment with enalapril, a non-thiol possessing ACE-I [195]. Another trial reported that captopril improved arthritis symptoms, clinical scores, plasma viscosity and CRP in patients with active RA [196]: captopril is a thiol containing ACE-I which has a close structural similarity to D-penicillamine, a drug that at the time was commonly used for treatment of RA. It was therefore difficult to attribute the disease modifying properties of captopril to ACE inhibition or to the presence of a thiol group. A later study investigated the effects of pentopril (CGS-13945), a non-thiol containing ACE-I, in 15 patients with active RA [197]. Even though the drug exerted its pharmacological effects, as shown by a modest BP drop and reduction in serum ACE activity, it produced little clinical or serological improvement, suggesting that the therapeutic benefit of captopril in RA probably lies in its thiol group rather than in its enzyme inhibition properties. Nevertheless, the dose of captopril which may be appropriate in RA is about five times lower than the required dose of D-penicillamine, suggesting an additional mode of action that remains unknown [198]. A randomised, double blind, placebo controlled, multicentre study assessing the effect of lisinopril 20 mg vs. placebo on rheumatoid disease activity score (DAS28) is also ongoing (Trial in Rheumatoid Arthritis of LISinopril-TRALIS) [199]. The main study objectives include the assessment of whether lisinopril has disease-modifying activity and whether it improves vascular health.

Effects on RA Synovitis

Even though the results regarding serum ACE activity in RA are controversial, synovial fluid ACE levels are universally increased in RA patients compared to patients with osteoarthritis (OA) [198, 200-203]. Elevated ACE activity has been demonstrated in blood monocytes [201], nodules [204], and synovial tissue (particularly endothelial cells, monocytes and fibroblast-like stromal cells) [200, 205] of patients with RA, and AT1 receptors are present in human synovial tissue [206]. However, levels of RAAS activity did not directly reflect membrane vascularity, monocyte or macrophage number, or the thickness of the lining layer [207]. Renin, which catalyzes the generation of angiotensin I from angiotensinogen seems to be both filtered from circulation [208] and locally generated in the inflamed synovium [203]. Furthermore, inactive renin is converted into the active form in the rheumatoid synovial fluid [203]. Increased levels of ACE can lead to increased locally-generated angiotensin II levels, which subsequently act on specific AT1 receptors present on the synovial microvessels and synovial stroma in the rheumatoid synovium [200] and modulate synovial perfusion [209]. The overall vasopressor effect of increased angiotensin II levels may lead to episodes of tissue hypoxia, which when followed by reperfusion may lead to formation of free radicals (ROS), well known mediators of cell damage in RA [210]. Further involvement of angiotensin II could be as a result of its ability to act as activator of the pro-inflammatory transcription factors NF-kB [211] and activator protein (AP-1) leading to up-regulation of TNF-a and IL-6 gene expression in macrophages [212, 213]; of chemoattractants for monocuclear cells, neutrophils, T and B lymphocytes to the synovium [214, 215]; and of growth factors regulating cellular growth, matrix synthesis, fibroblast proliferation, angiogenesis, and fibrosis [214-218], all of which may promote and exacerbate chronic inflammatory arthritis [219] (Fig. **4**).

However, increased ACE may also exert anti-inflammatory properties by inactivating bradykinin and substance P, both of which have been previously demonstrated to have pro-inflammatory effects in the inflamed joints [220]. In theory, ARBs may be preferential to ACE-I for patients with arthritis. ACE-I possess two potentially disadvantageous properties: they increase levels of bradykinin and substance P, both of which are pro-inflammatory, and their effect on reducing angiotensin II levels is limited by the presence of non-ACE dependent pathways (e.g. chymase) [221]. Since chymase is found in mast cells [222] and the latter are abundant in rheumatoid synovium [223], it seems likely that non-ACE-dependent angiotensin II production would be enhanced in this environment. This raises the possibility that AT1 receptor blockade may present a novel and more effective therapeutic target than ACE-I in the treatment of joint inflammation. In line with this, a recent study [224] has shown that ARBs suppressed the development of severe arthritis and joint destruction in the collagen-induced-arthritis model in mice, *via* attenuation of Th1 responses. Olmesartan, an ARB, inhibits the angiotensin II-induced NF-kB transcriptional activation [54], providing a possible explanation for these findings. Similar observations have been made in the collagen-induced arthritis mouse model, in a study showing that prophylactic or therapeutic treatment regimes with quinapril or candesartan reduced the severity of arthritis [225]. In parallel human *in vitro* experiments, ACE inhibition suppressed lipopolysaccharide-stimulated production of TNF-α by monocytes, an effect which is probably mediated by the documented ability of ACE-I to reduce NF-kB activation [169]. The latter suggests that autocrine production of angiotensin II is important in promoting human monocyte responses to other pro-inflammatory stimuli. Recently, it has also been shown that losartan at a dose of 15 mg/kg could inhibit both acute (carrageenan/kaolin) and chronic (Freund's complete adjuvant) arthritis models in rats by ≥50% [209]. This dose is much higher to that used therapeutically

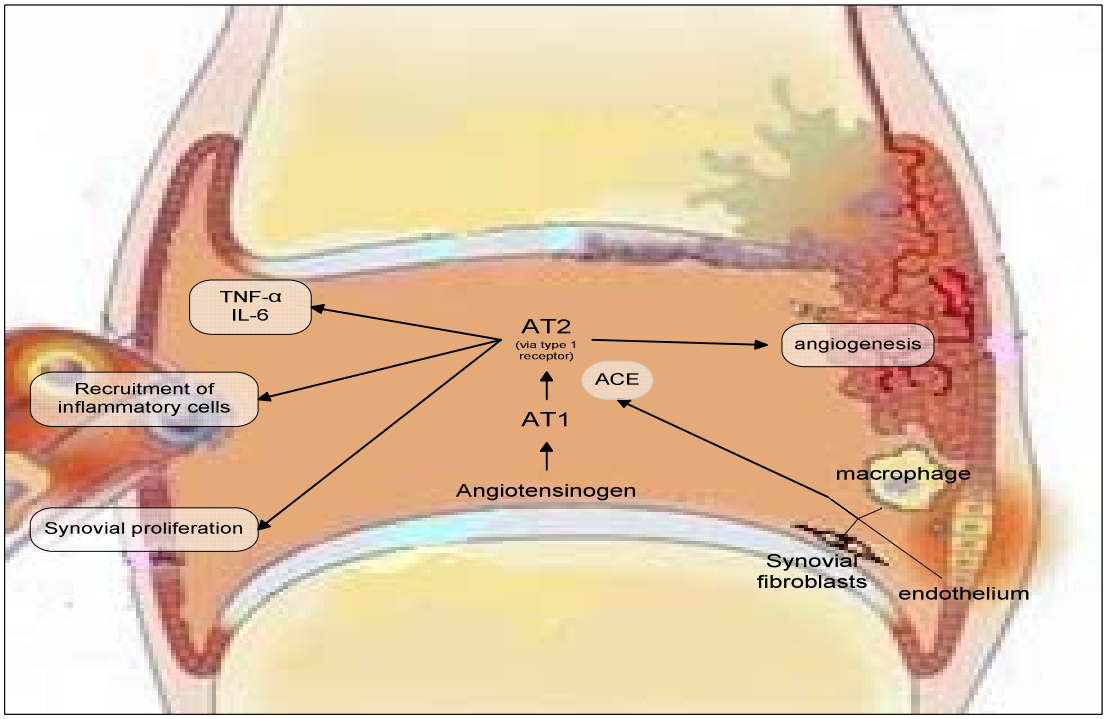

Fig. (4). The activated renin-angiotensin axis in the inflamed joint.

Synovial fluid ACE levels are universally increased in RA patients. Elevated ACE activity has been demonstrated in synovial tissue, particularly endothelial cells, monocytes and fibroblast-like stromal cells. Increased levels of ACE can lead to increased locally generated angiotensin II levels, which subsequently act on specific AT1 receptors present on the synovial microvessels and synovial stroma in the rheumatoid synovium and modulate synovial perfusion. Angiotensin II also activates of the pro-inflammatory transcription factors nuclear factor kappa B (NF-kB) and activator protein-1 leading to (**i**) up-regulation of TNF-a and IL-6 gene expression in macrophages, (**ii**) production of chemoattractants for monocuclear cells, neutrophils, T and B lymphocytes in the synovium and (**iii**) production of growth factors regulating cellular growth, matrix synthesis, fibroblast proliferation, angiogenesis, and fibrosis.

AT: angiotensin, ACE: angiotensin converting enzyme, TNF: tumor necrosis factor, IL: interleukin, TNF-α: tumor necrosis alpha, IL: interleukin.

in humans (1.5mg/kg), but it is possible that it may be tolerated by humans since ultra-high doses of irbesartan (900mg/day) were found to be safe in patients with type 2 diabetes mellitus [226].

ANTI-OBESITY DRUGS

Excess adiposity - especially abdominal - is one of the components of the MetS and a significant and independent predictor of future cardiovascular events [227]. Depending on the definition used, it is measured either as body mass index (BMI) or waist circumference [228]. However, adiposity can be much more accurately assessed using body composition analysis based on bioelectrical impedance, hydrostatic weighing, dual-energy x-ray absorptiometry, total body nitrogen, ^{40}K whole body counting, and urinary creatinine excretion thickness of partial subcutaneous fat, near-infrared rays, or ultrasound [229-234].

Adipose tissue, initially considered simply an energy reservoir, is now recognised as a metabolically active tissue. It secretes a number of bioactive proteins called adipokines or adipocytokines some of which, e.g.TNFα and IL-6, are implicated in local and systemic inflammation. These adipocytokines have also been implicated in insulin resistance and endothelial dysfunction, both major contributors to the development of CVD [235].

Consequently, the treatment of obesity is a cornerstone in the attempt to reduce CVD. Currently, lifestyle changes (i.e. diet and exercise) are considered to be the most effective means to reduce excess adiposity [236] but their uptake by the population at large does not appear to be sufficient to address the magnitude of the epidemic of obesity. Therefore pharmacological approaches are being developed, and some of them seem to have an additive effect to lifestyle modification, in terms of both weight loss and weight maintenance thereafter [236]. To date, three anti-obesity agents are available: Sibutramine, Orlistat and Rimonabant. Sibutramine is a centrally acting serotonin-norepinephrine reuptake inhibitor, which acts by inhibiting the reuptake of norepinephrine, serotonin and dopamine, thereby inducing satiety [237]. Orlistat is a pancreatic lipase inhibitor which reduces fat absorption from the intestine through inhibition of the hydrolysis of dietary fat into absorbable free fatty acids and monoacylglycerols [237]. Rimonabant is a cannabinoid type 1 receptor antagonist, which has been shown to reduce food intake *via* inhibition of the endocannabinoid system, which is known to play a role in the central and peripheral regulation of body weight and energy balance [238].

The results from two studies, investigating the effects of sibutramine-induced weight loss on inflammation, are conflicting [239,240]. Both administered sibutramine to obese individuals for a period of six months. One study found no effect of the treatment on adiponectin levels or CRP [239], whereas the other [240] detected significantly increased adiponectin levels and decreased CRP following treatment. The inconsistency between the two studies may lie in the magnitude of weight loss. Participants in the first study lost only 2% of their initial body weight. However, according to the findings of the latter study [240], a reduction of body weight greater than >5% is necessary for significant changes in adipocytokines to be seen.

The effects of weight-loss following Orlistat treatment on inflammation have been assessed in four studies [240-243]. Obese individuals were treated with Orlistat for six [240, 241, 243] or 12 [242] months. Irrespective of treatment duration, significant reductions in CRP [241-243], IL-6 [241,243], TNFa [241] and significant increases in adiponectin and leptin [242] were observed. However, one of the studies [240] was unable to trace statistically significant changes in the measured variables; yet, in that study, a trend towards increased adiponectin and decreased CRP following treatment was observed. Most likely, this is explained by the limited weight loss (i.e. <5% reduction in body weight) the participants experienced.

To date, there is no study in humans assessing the effects of Rimonabant on inflammation. However, there is evidence that administration of the drug over a 12 month period (in combination with diet) resulted in significant increases in serum adiponectin levels, irrespective of weight loss, suggesting the possibility of a direct impact of Rimonabant on inflammation [16]. This is further supported by two studies that investigated the anti-inflammatory effects of Rimonabant on rats [244,245]. The first [244] was conducted on lean and diet-induced obese rats with arthritis (induced by complete Freund's adjuvant). Results indicated that administration of Rimonabant reduced the global arthritic score and joint width in obese rats and also reduced thermal hyperalgesia and mechanical allodynia (i.e. pain) in both lean and obese rats, with a greater effect in the latter. The second [245] assessed the effects of Rimonabant on Escherichia coli lipopolysaccharide-induced plasma TNFα release. Results again indicated a favourable effect of the drug on inflammation as it inhibited overproduction of TNFα. The studies concluded that Rimonabant could potentially reduce pain as well as disease severity in inflammatory diseases [244, 245].

In RA adiposity has probably increased significance. Pro-inflammatory cytokines, such as IL-1 and IL-6, and TNFα are clearly implicated in the pathogenesis and progression of the disease [246, 247], and although the extent to which the adipose tissue produces these cytokines is still under intense investigation, it is widely accepted that inflammatory cytokine levels increase with increased body fat [7].

Almost two thirds of RA patients suffer from a condition termed rheumatoid cachexia, which is characterised by muscle wasting in the presence of stable total body weight [248]. As a result, RA patients present with significantly increased levels of body fat compared to healthy individuals of the same BMI [8]. This fact, coupled with the association of adiposity with cytokine production, lead to speculations that reductions in body fat of RA patients may positively affect RA disease activity, but direct evidence for this is currently lacking.

CONCLUSION

All three classes of drugs discussed in this review offer the promise of additive beneficial effects to conventional CVD risk factor reduction in patients with RA, through their anti-inflammatory, immunomodulatory potential, which may be useful both for control of cardiovascular comorbidity and rheumatoid disease modification (Fig. **5**). This needs to be

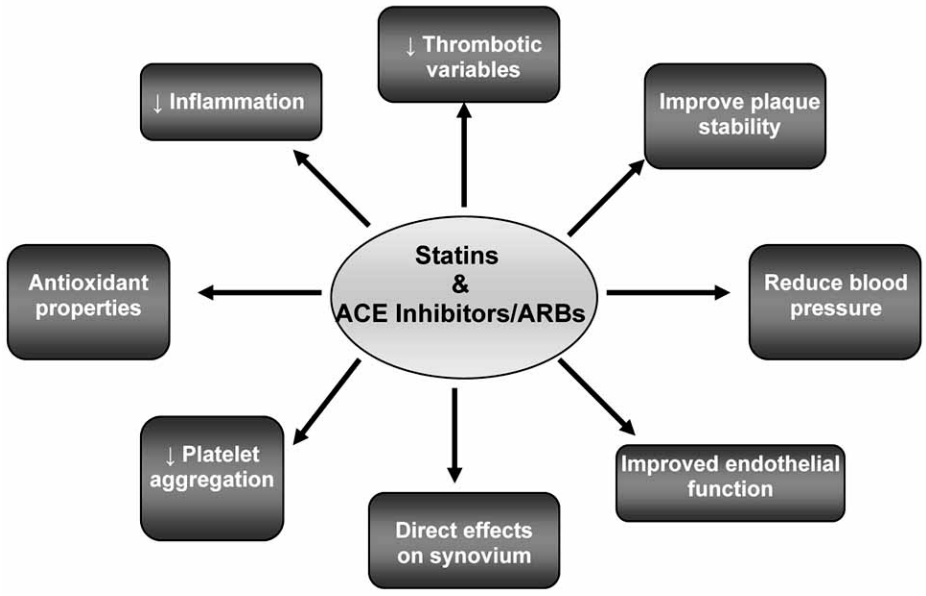

Fig. (5). Common beneficial effects of statins and ACE-I/ARBs.

ACE-I: angiotensin converting enzyme inhibitors, ARBs: angiotensin II type 1 receptor blockers.

tested in prospective RCTs designed specifically for the purpose.

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EXTENDED REPORT

Redefining overweight and obesity in rheumatoid arthritis patients

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Objectives: To assess whether body mass index (BMI) and body fat (BF) differ between rheumatoid arthritis (RA) patients, patients with non-inflammatory arthritis (osteoarthritis, OA) and healthy individuals, and whether disease specific measures of adiposity are required to accurately reflect BF in these groups.
Methods: 641 individuals were assessed for BMI (kg/m^2) and BF (bioelectrical impedance). Of them, 299 (174 RA, 43 OA and 82 healthy controls (HC)) formed the observation group and 342 (all RA) the validation group. RA disease characteristics were collected.
Results: ANOVA revealed significant differences between disease groups for BMI ($p<0.05$) and BF ($p<0.001$). ANCOVA showed that age accounted for the differences in BMI ($F_{1,294}=5.10$, $p<0.05$); age ($F_{1,293}=22.43$, $p<0.001$), sex ($F_{1,293}=380.90$, $p<0.001$) and disease ($F_{2,\ 293}=18.7$, $p<0.001$) accounted for the differences in BF. For a given BF, patients with RA exhibited BMI levels reduced by 1.83 kg/m^2 ($p<0.001$) compared to HC; there were no significant differences between OA and HC. A predictive model for BF was developed ($R^2=0.769$, $p<0.001$) and validated using limits of agreement Analysis against measured BF in the validation group (95%$LIM_{AG}=6.17$; CV = 8.94).
Conclusions: In individuals with RA, BMI cut-off points should be reduced by 2 kg/m^2 (that is, to 23 kg/m^2 for overweight and 28 kg/m^2 for obesity). The equation developed can be used to accurately predict BF from BMI in RA patients. These findings may be important in the context of the cardiovascular comorbidity of RA.

Excess body fat (BF) is a prominent health hazard[1] significantly contributing to the development of cardiovascular disease (CVD).[2] About two-thirds of patients who have had a myocardial infarction (MI) exhibit increased body weight.[3] Obesity increases the risk of coronary heart disease (CHD) through a number of different pathophysiological pathways, including insulin resistance, type 2 diabetes, hypertension and dyslipidaemia.[4 5]

Assessments for overweight or obesity include the calculation of body mass index[6] (BMI, in kg/m^2) or more accurate estimations of relative adiposity (BF percentage) through a number of techniques (for example, skinfold thickness, hydrostatic weighing and bioelectrical impedance).[7] BF estimations require sophisticated equipment and trained personnel, whereas BMI is easy to obtain and is widely used in the routine clinical setting.

In the general population, BMI of <25 kg/m^2, 25–30 kg/m^2 and >30 kg/m^2 indicate healthy, overweight, and obese individuals and associate with low, medium and high CVD risk, respectively.[8 9] However, BMI is only a proxy of body fat,[6] and over recent years its validity has been questioned.[3 7 10–13] Overweight as defined by BMI of >25 kg/m^2, has poor specificity in detecting excess body fat in healthy men and women of all ages[6] as well as in patients with coronary heart disease.[3] In specific subpopulations, such as people of Indian-Asian race,[10] women[11–13] and large size athletes,[7] new BMI cut-off points have been suggested that optimally reflect BF and may better predict CVD risk.

The weakness of BMI is that it does not distinguish between lean body mass and fat mass. Consequently people of similar stature and weight, but different muscle content, will have the same BMI but different BF levels. This tends to be more evident in individuals with low BMI levels.[6] Such limitations of the BMI may explain the better cardiovascular outcomes observed in overweight and mildly obese patients with established CHD compared to their normal weight counterparts, who may have proportionately more BF.[3] Therefore, although it is well established that CHD risk increases with advancing BMI levels,[9] global cut-off points may be misleading for several populations.

Central obesity poses a great risk for CVD.[13 15] Regional fat distribution, as measured by waist to hip ratio, has been proposed as a more accurate predictor of CHD risk than BMI.[14 15] Indeed, it has been suggested that obesity should be redefined based on waist to hip ratio instead of BMI, since waist to hip ratio is significantly associated with MI risk in most ethnic groups.[16] However, its predictive strength can be negatively affected by sex and overall body weight,[17] in a way that pear-shaped or obese individuals might have optimal waist to hip ratio but increased overall body weight. More research is necessary to identify the optimal definition of obesity as a predictor for CHD in the general population and specific subgroups.[18]

Patients with rheumatoid arthritis (RA) have an increased risk for CHD events.[19] RA is a chronic inflammatory disease which affects predominantly synovial joints, causing pain, swelling, stiffness and eventually irreversible damage and deformity, all of which may lead to significant reduction in physical activity. RA associates with increased mortality particularly from CHD,[19] most probably because of accelerated atherogenesis secondary to the metabolic and vascular effects of systemic inflammation.[20] Nearly two-thirds of all individuals with RA experience involuntary loss of fat-free mass and progressively increased fat mass in the presence of stable or even slightly decreased weight, a condition referred to as

Abbreviations: ANCOVA, analysis of co-variance; ANOVA, analysis of variance; BF, body fat; BMI, body mass index; CHD, coronary heart disease; CVD, cardiovascular disease; DAS, disease activity score; HC, healthy controls; MI, myocardial infarction; LIM_{AG}, limits of agreement; OA, osteoarthritis; RA, rheumatoid arthritis

Table 1 Demographic and disease characteristics of all volunteers (mean (SD))

	Observation group						Validation group	
	Male (n = 110)			Female (n = 189)			Male	Female
	RA	OA	HC	RA	OA	HC	RA	RA
Number	56	15	39	118	28	43	99	243
Age	60.6 (11.8)**	56.7 (13.3)*	45.1 (13.3)	59.6 (12.2)** †	52.8 (12.5)*	46.8 (11.5)	62.1 (11.6)	61.7 (11.9)
Height	173.6 (7)*	171.3 (6.7)*	177.3 (6.7)	159.1 (6.5)**	161 (5)	163.6 (6.9)	174 (6.8)	160.4 (6.7)
Weight	83.6 (13.3)	78.4 (14.8)	80.9 (11.4)	68.6 (15)	70.8 (16.5)	68.1 (16.3)	82.7 (15.8)	70.2 (14.4)
BMI	27.7 (4.3)*	26.8 (4.7)	25.7 (3)	26.9 (5.7)	27.2 (5.7)	25.4 (5.5)	27.3 (4.4)	27.3 (5.3)
BF	28.7 (7.7)**	24.8 (7.9)*	19.2 (5.2)	38.3 (7.3)** †	35.2 (8.5)	32.1 (8.2)	27 (6.4)	38.3 (7.1)
Trunkal fat	30.5 (8)**	26.6 (8.9)*	21.4 (6)	35.7 (8.6)** †	31.6 (9.6)	29.1 (8.7)	27.4 (7.7) §	35.4 (8.1)
DAS28	4.2 (1.2)			4.3 (1.4)			4.1 (1.4)	4.3 (1.4)
ESR (mm in 1st hour)	23.2 (18.5)			26 (22.1)			25.3 (21.5)	30 (26.3)
CRP	15.6 (15)			15.8 (14.9)			16.8 (18.6)	17.6 (23.6)
Disease duration	11.4 (10.2)			11.3 (9.9)			12.5 (11)	13.2 (11)

RA, rheumatoid arthritis; OA, osteoarthritis; HC, healthy controls; BMI, body mass index; BF, body fat; DAS28, disease activity score-28; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.
One way ANOVA: *Significant difference compared to HC ($p<0.05$).
**Significant difference compared to HC ($p<0.001$).
†Significant difference compared to OA ($p<0.05$).
§Significant difference compared to experimental RA group ($p<0.001$).

rheumatoid cachexia.[21] The exact mechanisms causing rheumatoid cachexia remain undetermined, but muscle loss due to systemic inflammation and reduced physical activity may both contribute.[22]

We hypothesised that for a given BMI, RA patients exhibit significantly higher proportions of fat mass than healthy individuals, or even than patients with movement restriction due to a non-inflammatory arthritis, such as osteoarthritis (OA). The possible consequences of this, in the context of the increased CVD mortality in RA, are obvious. In the present study we aimed to investigate whether BMI and BF differ according to arthritic disease (OA vs RA) and within RA according to disease state (for example, active vs inactive, early vs established disease). We also developed and validated RA specific BMI cut-off levels and algorithms to calculate BF from BMI.

METHODS

Participants

Consecutive patients attending routine rheumatology or orthopaedic outpatient clinics at the Dudley Group of Hospitals NHS Trust, UK, and healthy controls (hospital and university staff) were invited to participate. The study had local research ethics committee approval by the Dudley ethics committee, and all volunteers provided informed consent. The observation group (n = 299) included 174 volunteers with RA (1987 revised American Rheumatism Association criteria[23]), 43 with OA of the hip[25] or knee,[26] and 82 healthy controls (individuals who by self report did not have any known clinical conditions and were taking no medication). The validation group (n = 342) consisted of RA patients only. Demographic and disease characteristics from all subjects appear in table 1.

Assessments

All volunteers were subjected to the same data collection procedures overseen by the same trained investigators. Specifically, standing height was measured to the nearest 0.5 cm on a Seca 214 Road Rod portable stadiometer. Body composition was assessed by bioelectrical impedance, using a Tanita BC-418 MA Segmental Body Composition Analyzer, which incorporates eight tactile electrodes (Tanita Corporation,

Table 2 BMI and BF of RA patients (observation group) according to categorisation based on their disease characteristics

		BMI		BF	
Disease characteristics	Categories	Male	Female	Male	Female
DAS28 (DAS28 score)	Remission (<2.6)	27.2 (3.46)	27.2 (5.6)	26.5 (7.6)	39.5 (6.7)
	Mild (2.7–3.2)	28 (4.3)	27.3 (4.6)	28 (6)	39.3 (6.6)
	Moderate (3.3–5.1)	27.8 (4.5)	27 (5.3)	27.4 (6.8)	37.3 (7.7)
	High (>5.1)	25.3 (5.5)	27.3 (5.5)	26.1 (5.6)	37.7 (7.2)
ESR (mm in 1st hour)	Normal*	27.9 (4.4)	26.9 (4.8)	27.1 (7.2)	38.3 (6.3)
	High	26.4 (4.6)	27.6 (6.1)	26.7 (5.9)	37.6 (8.9)
CRP (mg/l)	Low (<3)	26.5 (2.4)	28.3 (6.2)	25.9 (5.4)	38.5 (8.7)
	Normal (3–8)	27.8 (4.7)	26.5 (4.7)	26.7 (8)	37.6 (6.6)
	High (>8)	26.9 (4.6)	27.6 (5.7)	27.3 (5.8)	38.3 (7.9)
Disease duration (years)	Early (<3)	26.4 (5)	26.1 (5)	26.4 (7.9)	37.9 (8.3)
	Established (3–10)	28.8 (4.1)	27.8 (5.7)	27.8 (6.3)	38.2 (7.5)
	Longstanding (>10)	26.8 (4.4)	27.1 (5.1)	27.7 (5.7)	38.8 (6.7)
Rheumatoid factor	Positive	26.6 (3.6)	27.2 (5.7)	25.1 (6.7)	38.3 (7.3)
	Negative	27.5 (5)	27.1 (5.1)	27.7 (6.4)	37.9 (7.3)
Corticosteroid administration	Yes	27.1 (4.4)	27.3 (5.3)	26.2 (6)	38.1 (7.4)
	No	24.5 (4.9)	26.7 (5.3)	27.8 (7.3)	37.7 (7)

DAS28, disease activity score-28; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.
For all differences between groups: $p>0.05$.
*Normal ESR: <50 years: male <15, female <20.
>50 years: male <20, female <30.

Tokyo, Japan). This apparatus measures total body mass and assesses body composition in terms of percentage body fat, fat mass, fat free mass and total body water, as well as fat distribution in different body segments (abdominal and peripheral fat) and has a standard error of <3.[26] After initial manual entry of their demographic details, participants stood bare footed on the analyser and held the handgrips provided until the apparatus printed the results. BMI (kg/m^2) was calculated on the basis of measured height and weight. In RA patients, contemporary serological inflammation and clinical disease activity were assessed by the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) (using routine laboratory procedures) and the disease activity score-28 (DAS28).[27] Disease duration was recorded from review of the patients' hospital notes.

Data management and analysis

Data were inserted in a purpose designed spreadsheet (Microsoft Excel 2003) and audited for accuracy weekly. They were exported for analysis to the Statistical Package for Social Sciences version 11.0 (SPSS Inc, Chicago, IL, USA). Preliminary evaluation of the variables using a Kolmogorov-Smirnov test of normality revealed that none of them required logarithmic transformation to reach normality. Means (SD) were calculated for all variables.

The method of analysis was to define either BMI or BF as the dependent variable and then to incorporate all other known parameters thought to influence these measures of adiposity as either factors in an ANOVA or factors with covariates in an ANCOVA. Factors included sex and disease status (RA, OA and HC) while age, disease activity and duration, and serological inflammation were entered as continuous covariates. The initial ANCOVA analysis incorporated all these factors and covariates, but only those found to be significant were subsequently retained and reported in the prediction equation model below.

Within the RA population of the observation group, correlations of disease activity (DAS28, ESR, CRP) and disease duration with BMI and BF were obtained for each sex. RA patients were also subgrouped according to their clinical disease activity (DAS remission <2.6, mild 2.7–3.2, moderate 3.3–5.1, high >5.1[27]), serological inflammation (ESR[28] and CRP[29]), disease duration (early <3 years, established 3–10 years, longstanding >10 years), rheumatoid factor positivity (ever), or corticosteroid administration (yes/no ever): differences between these subgroups in relation to BMI and BF were assessed using ANCOVA (table 2). The level of significance was set at $p<0.05$.

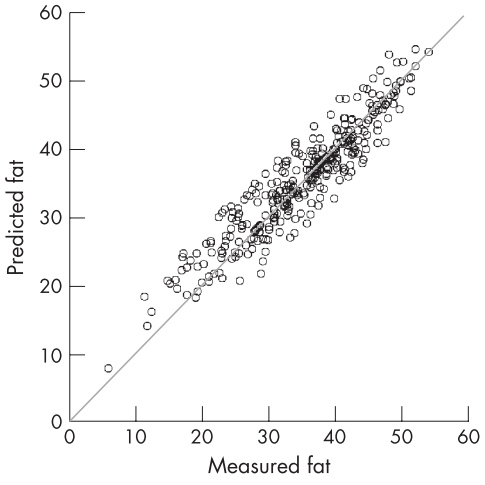

Figure 1 Agreement between predicted and measured fat in patients with RA. Body fat was measured by bioelectrical impedance using a Tanita BC-418 MA Segmental Body Composition Analyzer. Predicted fat was assessed using the formula: BF = 4.273 + sex − 0.719 + 0.108 × age + 1.059 × BMI. 95% limits of agreement were 6.17 with a coefficient of variation of 8.9.

The external validity of the predictive model was tested with the limits of agreement (LIM_{AG}) method[30] against BF of the validation group. The limits of agreement were obtained as follows:

(1) We calculated the mean (d) and the standard deviation (s) of the differences that indicate the level of bias and the random variation between the two measures of BF (that is, the predicted BF and measured BF of the validation group, respectively).
(2) Provided the differences are normally distributed, the 95% limits of agreement are given by: $d \pm (1.96 \times s)$.

Bland and Altman[30] argue that, provided that differences within these limits are not clinically important, the two measurement methods can be used interchangeably.

RESULTS

Observation group

Within the RA population of the observation group, no significant correlations were found between DAS28, ESR, CRP, disease duration and BMI or BF. Similarly, when RA patients were grouped according to these variables as well as rheumatoid factor positivity and corticosteroid use, no significant differences for BMI and BF were observed ($p>0.05$ in all cases, see table 2).

Between the different disease groups, one way ANOVA revealed significant differences in BMI ($p<0.05$) and BF ($p<0.001$; table 1): RA males had higher BMI and BF (including trunkal fat) than HC males, and RA females had higher BF than HC females, even though their BMI did not differ significantly. ANCOVA revealed that BMI differences between the groups were mainly the result of the significant effect of the covariate age ($F_{1,294} = 5.10$, $p < 0.05$) and not because of disease ($F_{2,294} = 1.00$, $p > 0.05$), sex ($F_{1,294} = 0.59$, $p > 0.05$) or their interactions.

ANCOVA also revealed that RA and OA patients exhibited lower BMI levels than their HC for a given BF. However, differences were only significant for the RA patients (RA: −1.826 kg/m^2 ($p<0.001$); OA: −0.352 kg/m^2 ($p>0.05$)). BMI was significantly ($p<0.001$) predicted by age, disease, sex and BF ($R^2 = 0.58$).

When BF was adopted as the dependent variable, ANCOVA identified significant differences between disease groups ($F_{2,293} = 18.70$, $p<0.001$) and sex ($F_{1,293} = 380.90$, $p<0.001$) together with a significant covariate, age ($F_{1,293} = 22.43$, $p<0.001$). The contribution of BMI as a covariate in this analysis was also significant ($F_{1,293} = 370.74$, $p<0.001$). For a given BMI, RA patients exhibited significantly increased levels of BF (4.273, $p<0.001$) compared to healthy controls. The difference for OA patients was non-significant (1.648, $p>0.05$). The variation of BF was predicted by age, gender, BMI, and disease type ($R^2 = 0.769$, $p<0.001$). This was only very slightly improved (for RA) by the addition of RA disease duration ($F_{1,293} = 0.340$, $p>0.05$) in the equation (from 76.9% to 77.1%), so we did not include this variable in the final model. The predictive model obtained from this analysis is:

- BF = disease status + sex − 0.719 + 0.108 × age + 1.059 × BMI
  - Disease status: RA = 4.273, OA = 1.648, HC = 0.
  - Sex: male = −11.294, female = 0.

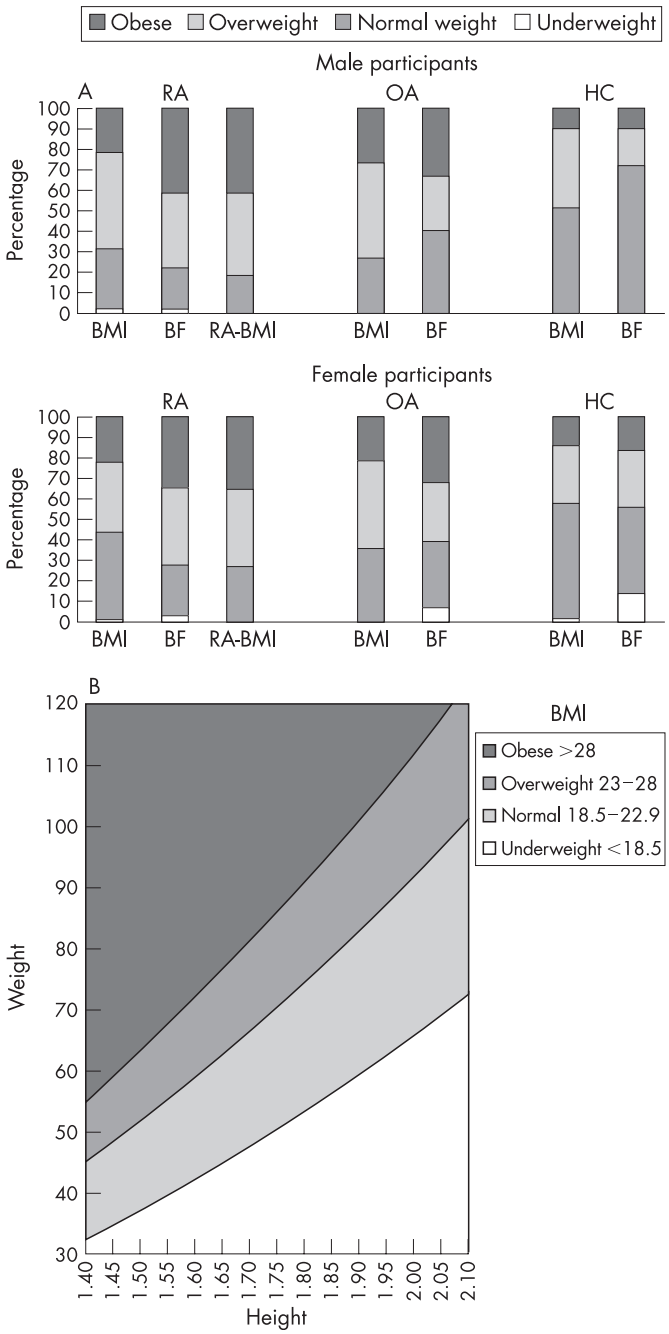

Figure 2 (A) Classification of male (top) and female (bottom) participants into obese, overweight, normal and underweight groups according to currently accepted BMI cut-off points (BMI), body fat content (BF) and RA specific BMI cut-off points (RA-BMI). Accepting BF as the most accurate assessment of body fatness, currently accepted BMI cut-off points misclassify a significant proportion of both males and females with RA (notice the difference in the respective bars). This misclassification is corrected when the proposed RA specific BMI cut-off points are applied. RA, patients with rheumatoid arthritis; OA, patients with osteoarthritis; HC, healthy controls; BMI, classification according to existing body mass index (BMI) cut-off points of 25 kg/m^2 for overweight and 30 kg/m^2 for obesity; BF, classification according to age and sex specific cut-off points for body fat percentage; RA-BMI, classification according to the proposed RA specific BMI cut-off points of 23 kg/m^2 for overweight and 28 kg/m^2 for obesity. (B) BMI chart developed specifically for patients with RA. Values were calculated using the formula: BMI = weight (in kg)/height^2 (in metres) for the rheumatoid arthritis specific BMI levels identified in the present study (23 kg/m^2 for overweight, 28 kg/m^2 for obesity). The generally accepted lower threshold for normal BMI (18.5 kg/m^2) was not altered.

Validation group

To establish external validity of our predictive model, we assessed its agreement with the measured BF in 342 patients with RA. Preliminary analyses for LIM_{AG} revealed no heteroscedasticity, thus the LIM_{AG} can be reported as absolute measurements.[30] Our analyses suggested that the bias of our prediction is 0.4 (that is, our model overpredicts BF by 0.4) with a standard error of 3.2 (95% $LIM_{AG} = 6.17$, coefficient of variation = 8.9; fig 1). The difference is statistically significant ($t = 2.3$, $p<0.05$), but the coefficient variation (CV = 8.9) is within acceptable limits.

RA specific BMI cut-off levels

The fact that patients with RA exhibited increased BF values for a given BMI compared to HC suggested that BMI cut-off points in the RA population would be more appropriate if they were reduced by approximately 2 kg/m^2 (to 23 kg/m^2 and 28 kg/m^2 for overweight and obesity, respectively). We therefore compared the proportions of subjects in each group that would be correctly classified as overweight or obese using the widely accepted BMI cut-offs of 25 kg/m^2 and 30 kg/m^2 vs the proposed (for RA) 23 kg/m^2 and 28 kg/m^2 vs the age and sex specific cut-off points of measured BF. This analysis showed that 9% of male and 15% of female RA patients would be misclassified as of normal weight based on traditional BMI cut-offs. Such misclassification was not a problem either for OA or HC, where if anything, BMI overestimated BF. Application of the proposed RA specific BMI cut-offs of 23 kg/m^2 and 28 kg/m^2 corrected this misclassification (fig 2A). A modified, RA specific BMI chart for the classification of patients with RA into underweight, normal, overweight and obese categories was developed and is provided in figure 2B.

DISCUSSION

The validity of BMI as an acceptable measure of overweight or obesity, and as an accurate reflection of body fat (BF) content, has been repeatedly questioned and the need for population specific BMI cut-off points has been highlighted.[7 10–13] Ideally, individualised assessment of BF should be pursued in the clinical setting, as BF percentage is a more reliable measure of fatness than BMI, at least in the general population.[31] Indeed, our data indicate that only 58% of the variance in BMI can be predicted, as opposed to 77% in BF. BF in vivo can be determined via a number of methods such as underwater weighing, dual energy x ray absorptiometry, total body water, total body nitrogen, ^{40}K whole body counting and urinary creatinine excretion.[32–34] BF can also be estimated from the thickness of partial subcutaneous fat, near infrared rays and ultrasound.[35] However, none of these methods can be practically used in the routine clinical setting as they require sophisticated apparatus and specialised personnel.[33]

In recent years, a bioelectrical impedance method for the estimation of BF in different populations has become popular and widely recommended, as it is reliable, objective, practical, relatively inexpensive and does not require highly trained personnel.[32 33] The validity of this method has been confirmed in various studies.[32 36–39] Devices with eight tactile electrodes using single frequency electrical current, similar to the one used in this study, generate highly reproducible measurements of total BF and segmental fat distribution.[40] Their correlation with the "gold standards" of dual energy x ray absorptiometry and hydrostatic weighing is 0.90 and 0.80, respectively, with a standard error of around 3.0, producing a coefficient of variation of <10%.[33] This suggests that bioelectrical impedance measurements (especially when using eight electrodes) are valid and suitable for body composition studies.[32 39 40] Patients

are usually happy to undergo such a measurement because of its simplicity and similarity to normal weighing.

In the absence of the necessary equipment or expertise, the predictive model presented here can be used to easily calculate BF of RA patients from BMI. The cross validation of this predictive model in patients with RA is reassuring. Even though there was a statistically significant difference between the measured and the predicted BF, closer examination of the means indicates that this difference is at a level of less than 0.5% of BF with a coefficient of variation of <10%. The statistical significance of such a small difference can be attributed to the very large number of the validation group and is clinically not significant. However, the parts of the equation referring to OA patients and healthy individuals need further prospective validation in sufficiently large samples of the relevant populations.

BMI remains the most commonly used indicator of body fatness in the clinical setting, and the cut-off points of 25 kg/m^2 and 30 kg/m^2 (for overweight and obesity, respectively) used for the general population are also routinely applied in RA patients. This study shows that application of these BMI cut-off points misclassified 9% of male and 15% of female RA patients in terms of actual body fatness. For a given BMI, RA patients exhibited an average 4.3% increase in BF compared to healthy controls. In contrast, for the same level of BF, RA patients had BMI values almost 2 kg/m^2 lower than those of healthy controls. We propose that BMI cut-off points in the RA population should be lowered to 23 kg/m^2 (from 25 kg/m^2) for overweight, and 28 kg/m^2 (from 30 kg/m^2) for obesity. The lowest limit for normal BMI (that is, 18.5 kg/m^2) should remain unaltered, as low BMI levels have been related to increased cardiovascular risk in patients with RA.[41 42] We also provide a chart for the classification of RA patients in normal, overweight and obese categories according to these BMI cut-offs, for use in the routine clinical setting (fig 2B).

The most likely explanation for the BMI and BF differences observed in RA is rheumatoid cachexia associated with the chronic inflammatory response, given that such differences were not as prominent in OA. RA patients experience accelerated involuntary loss of fat-free mass, predominantly in the skeletal muscle, in excess of what is normally expected as a result of the ageing process.[43] Although the underlying mechanisms for rheumatoid cachexia remain unknown, possible contributing factors include the overproduction of inflammatory cytokines such as tumour necrosis factor α and interleukin 1β.[43 44] Our subanalyses within the RA population revealed that neither BMI nor BF were associated with current clinical or serological disease activity, seropositivity for rheumatoid factor (which tends to associate with more severe disease) or corticosteroid administration. This is not totally surprising as disease activity may vary within small periods of time, depending on medication and the disease itself, whereas changes in body composition are longer term processes. On the other hand, disease duration appeared to be of some importance. It is possible that most alterations in body composition of RA patients occur in the first few years of the disease, as it has previously been reported,[21] irrespective of disease characteristics or medical treatment.

The results of the present study are reminiscent of the observations made for Asian populations, which have significantly higher CVD risk than white people: BF in Asians has been found to be 3–5% higher than that of white people with similar BMI, whereas BMI was 3–4 kg/m^2 lower than that of white people with similar BF.[32] Differences in body build (trunk to leg length ratio and slenderness) and in muscularity have been suggested as possible explanations for these discrepancies. As a result, new cut-off points for Asian populations have been set at 23 kg/m^2 and 27 kg/m^2 for overweight and obesity, respectively,[10] and have been shown to be more sensitive in identifying Asians at increased risk for CVD.[45]

In our participants, lowered BMI cut-off points would reflect an average reduction of 5–6 kg, or 8%, in the ideal weight (the weight one should have in order to be below the BMI cut-off for overweight). Such reductions in body weight are likely to lead to physiological benefits in the cardiovascular system: in the general population, even a 5% reduction of body weight is known to favourably affect most classic CVD risk factors.[46 47]

The reduced BMI cut-off points for RA suggested here may be of significance both for the management of individual patients and for further research into the cardiovascular morbidity and mortality of RA. In the clinical arena, the reduction of these thresholds would identify an additional 10–15% of people with RA as overweight or obese, and may trigger closer scrutiny for other CVD risk factors and appropriate intervention, if necessary. Moreover, obesity, defined by the BMI, is one of the WHO criteria for the metabolic syndrome.[46] Aggressive identification and reduction of classic CVD risk factors in patients with RA is an obvious strategy for reducing the increased cardiovascular mortality of this disease.[19] From the research perspective, the new thresholds may trigger re-analysis of previously published cohorts or further analysis of prospective cohorts as to the importance of body fat as a predictor of CVD in RA and its association with other individual risk factors.

We conclude that, in the clinical setting, body fatness of RA patients should be evaluated based on the BMI cut-off points of 23 kg/m^2 for overweight and 28 kg/m^2 for obesity. In the absence of specialised equipment, if necessary, BF of patients with RA can be estimated from BMI using the equation provided.

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ORIGINAL ARTICLE

Underweight and obese states both associate with worse disease activity and physical function in patients with established rheumatoid arthritis

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Abstract Obesity is characterised by low-grade inflammation and could potentially affect disease activity and severity in patients with rheumatoid arthritis (RA). Body mass index (BMI), body fat (BF), erythrocyte sedimentation rate, C-reactive protein, disease activity score 28, physical function (health assessment questionnaire) and presence of erosions and joint surgery were assessed in 294 (female=219) volunteers with established RA [age 63.3 (56.2–69.6); disease duration 13 (7–20) years]. Smoking status, rheumatoid factor and anti-cyclic citrullinated peptide positivity were also assessed. BMI and BF independently associated with disease characteristics. Compared to normal-weight patients, underweight and obese had higher C-reactive protein (p=0.046) and physical dysfunction (p=0.034). BMI or BF did not associate with presence of erosions or joint surgery. In patients with established RA, both very low and very high BMI and BF associate independently with increased disease activity and physical dysfunction; however, this does not seem to associate with presence of erosions or joint surgery. Further longitudinal studies are required to address this apparent dissociation.

Keywords Body weight · Body mass index · Obesity · Percentage body fat · Rheumatoid arthritis outcome

Introduction

Rheumatoid arthritis (RA) is the commonest inflammatory arthritis [1, 2]. It affects predominantly the synovial joints,

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which are infiltrated by chronic inflammatory cells producing cytokines such as tumour necrosis factor-α (TNF-α) and interleukin (IL)-1 [1]. Permanent joint damage and functional decline usually ensue [3], so the efforts of the scientific community have focused on symptom control and limitation of joint damage [4].

An indirect effect of RA is alteration in body composition. Almost two thirds of RA patients suffer from a condition termed rheumatoid cachexia, which is characterised by muscle wasting in the presence of stable total body weight [5]. As a result, RA patients present with significantly increased levels of body fat compared to healthy individuals of the same body mass index (BMI) [6].

Adipose tissue, initially considered to be simply an energy reservoir, is now recognised as a metabolically active tissue. It secretes a number of bioactive proteins called adipokines or adipocytokines, including TNF-α and IL-6 [7]. This could potentially result in more active disease in obese RA patients. However, studies in patients with early RA, of up to 3 years duration, surprisingly suggest that obesity may protect against joint damage [8–10]. In contrast, studies in unselected (for disease duration) RA patients suggest that obesity leads to worse quality of life [11], indicating that its potential protective effects in early RA are diminished or reversed later on in the course of the disease. The present study aimed to add information in the field by assessing the associations of body weight and body fat with RA characteristics in patients with well-established disease.

Materials and methods

Participants

Consecutive patients with RA (1987 revised American College of Rheumatology criteria [12]) of more than 3 years duration since symptom onset, attending routine rheumatology clinics at the Dudley Group of Hospitals NHS Trust, UK, were invited to participate. The study had Local Research Ethics Committee approval, and all volunteers provided informed consent conforming to the declaration of Helsinki. A total of 294 (male=75, female=219) volunteers were assessed, reflecting the classical male-to-female ratio (i.e. 1:3) of RA [13]. Their demographic and disease characteristics appear in Table 1.

Table 1 Demographic and disease characteristics of all volunteers [median (interquartile range) or percentage of positives]

	Male	Female
N	75	219
Age (years)	62.1 (54.2–69.7)	62.1 (55–68.1)
Height (cm)	173.0 (168–178)	160 (155.5–164)
Weight (kg)	83.6 (74.3–93.6)	70 (60.9–80.7)
BMI (kg/m^2)	27.6 (25.8–30.3)	26.9 (24.1–31.3)
BF (%)	28.8 (24.1–31.7)	38.9 (34.5–43.2)
ESR (mm/h)	18.5 (6.8–31)	21 (10–36)
CRP (mg/L)	12 (6–22.3)	8.0 (5–20)
DAS28	4 (3.3–4.9)	4.1 (3.3–5.1)
HAQ	1.4 (0.5–2)	1.6 (0.9–2.3)
Presence of erosions (%)	72.8	60.9
Knee surgery (%)	6.7	8.7
Hip surgery (%)	5.3	5.5
Wrist/hand surgery (%)	10.7	9.1
Elbow surgery (%)	1.3	2.3
Shoulder surgery (%)	2.7	1.6
Neck surgery (%)	4	2.7
Any surgery (%)	30.7	29.9
Disease duration (years)	14.5 (7.8–20)	12 (7–22)

N number, *BMI* body mass index, *BF* body fat percentage, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *DAS28* disease activity score 28, *HAQ* health assessment questionnaire

Assessments

All volunteers were subjected to the same data collection procedures overseen by the same trained personnel. Specifically, standing height was measured to the nearest 0.5 cm on a Seca 214 Road Rod portable stadiometer (Seca GmbH & Co. Kg., Hamburg, Germany). Body weight and composition were assessed using a Tanita BC-418 MA Segmental Body Composition Analyser (Tanita Corporation, Tokyo, Japan). After initial manual entry of their demographic details, participants stood bare-footed on the analyser and held the handgrips provided until the apparatus printed the results. This apparatus measures total body mass and assesses body composition in terms of percentage body fat (BF), fat mass, fat-free mass and total body water, as well as fat distribution in different body segments (abdominal and peripheral fat) and has a standard error of <3 [13, 14]. Body mass index (BMI in kilogramme per square metre) was calculated on the basis of measured height and weight. Waist circumference was measured to the closest 0.5 cm using a Seca 200 Circumference measuring tape (Seca GmbH & Co. Kg., Hamburg, Germany).

Positivity for rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibodies was assessed in serum using enzyme-linked immunosorbent assay microplate techniques (DIASTAT™, Axis-Shield Diagnostics Ltd., Dundee, UK). For RF, patients with a concentration of >20 units per millilitre were considered positive; for anti-CCP, patients with a concentration of >5 units per millilitre were considered positive.

Erythrocyte sedimentation rate (ESR) was measured using a Starrsed compact device (Mechatronics BV, Netherlands). A total of 10 ml of undiluted blood, anti-coagulated with EDTA, was inserted in a vertical tube. The sedimentation (in millimetres) of the red blood cells within 1 h gives the value

of ESR. C-reactive protein (CRP) was measured in blood serum with a Vitros® 5.1 FS chemistry system (Johnson and Johnson Inc., Langhorne, PA, USA).

Clinical disease activity and physical function were assessed by the Disease Activity Score-28 (DAS28) [15] and the Anglicised version of the Stanford Health Assessment Questionnaire (HAQ) [16], respectively. HAQ, a surrogate of cumulative disease activity [17], served as the primary outcome measure of the present study. X-rays of hands and wrists were independently assessed by two rheumatologists for presence of erosions; in case of disagreement (nine in total), X-rays were jointly reviewed and a consensus opinion reached. Information on disease duration, smoking status and previous joint surgery (presence or absence) were obtained from patient interview and confirmed by reviewing the patients' hospital notes.

Data management and analyses

Data were inserted in a purpose-designed spreadsheet (Microsoft Excel 2003) and audited for accuracy weekly. They were exported for analysis to The Statistical Package for Social Sciences version 15.0 (SPSS Inc. Chicago, IL, USA). The Kolmogorov–Smirnov test of normality was used to assess dispersion of the variables.

Spearman's correlations were used to assess the association of weight, BMI and BF with disease activity and physical function (i.e. ESR, CRP, DAS28, HAQ). These associations were subsequently adjusted for age, gender, smoking status, RF and anti-CCP positivity and disease duration using multivariable analyses.

Thereafter, binary logistic models, with backward elimination of statistically insignificant variables, were used to test the associations of weight, BMI and BF with the presence of erosions and joint surgery. For joint surgery, independent examination of the association for each joint area (i.e. neck, shoulder, elbow, hand and wrist, hip, knee, ankle and forefoot) was pursued. The total number of joint operations was calculated and its association with BMI and BF was tested using multinomial regression. Results were standardised for age, gender, smoking status, RF and anti-CCP positivity and disease duration.

Finally, participants were categorised according to RA-specific BMI [6] into four distinct subgroups (i.e. underweight, normal weight, over-weight and obese). Analysis of variance (ANOVA) was used to assess differences between groups for disease activity and physical function (i.e. ESR, CRP, DAS28, HAQ). Analysis of covariance was used to assess the independence of these associations from age, gender, smoking status, RF and anti-CCP positivity and disease duration. BMI groups were also subjected to a cross-tabulation with presence of erosions and total number of operations and chi-squared analyses were performed.

Dispersion of data is reported as median (interquartile range) due to their not-normal distribution pattern. Results of the logistic models are reported as odds ratio with 95% confidence intervals (OR, 95% CI). Statistical significance was set at $p<0.05$.

Results

Weight correlated significantly only with CRP ($r=0.161$, $p=0.002$). BMI correlated significantly with ESR ($r=0.145$, $p=0.012$), CRP ($r=0.178$, $p=0.002$) and HAQ ($r=0.117$, $p=0.044$). Similarly, BF correlated significantly with ESR ($r=0.168$, $p=0.005$) and HAQ ($r=0.179$, $p=0.003$). After adjustment for age, gender, smoking status, RF and anti-CCP positivity and disease duration, the association of weight with CRP was lost. BMI retained its association only with ESR ($F_{1,\ 290}=7.567$; $p=0.006$) and HAQ ($F_{1,\ 290}=4.059$; $p=0.045$) whereas BF was found to associate with ESR ($F_{1,\ 290}=5.767$; $p=0.017$), CRP ($F_{1,\ 290}=4.162$; $p=0.042$) and HAQ ($F_{1,\ 290}=7.726$; $p=0.006$). The association of BF with DAS28 was borderline non-significant ($F_{1,\ 290}=3.888$; $p=0.055$).

Binary logistic regression showed no association of either weight, BMI or BF with the presence of erosions. Subsequently, the same analyses revealed an inverse association of BMI with neck surgery (OR=0.781, 95% CI 0.637–0.958; $p=0.018$) and a positive association of BF with total knee replacement (OR=1.146, 95% CI 1.094–1.201; $p=0.046$), but no other associations were found. Multinomial regression models showed no association of either BMI or BF with the total number of operated joints.

Following patient grouping according to BMI into underweight, normal weight, over-weight and obese, ANOVA demonstrated significant differences in CRP ($p=0.046$) and HAQ ($p=0.034$) between the groups: patients who were either underweight or obese had significantly worse CRP and HAQ than those who had normal weight, in an almost U-shaped mode (Fig. 1). A similar trend was seen with ESR and DAS28 also, but the differences were not significant ($p=0.095$ and $p=0.063$, respectively; Fig. 1). Chi-squared analyses failed to identify any differences between BMI subgroups for either the presence of joint erosions or total number of operations.

Discussion

This study aimed to identify possible associations between weight, BMI and/or body fat with RA activity and severity in patients with established disease of more than 3 years duration. Weight did not associate with any of the studied variables. However, BMI significantly associated with ESR

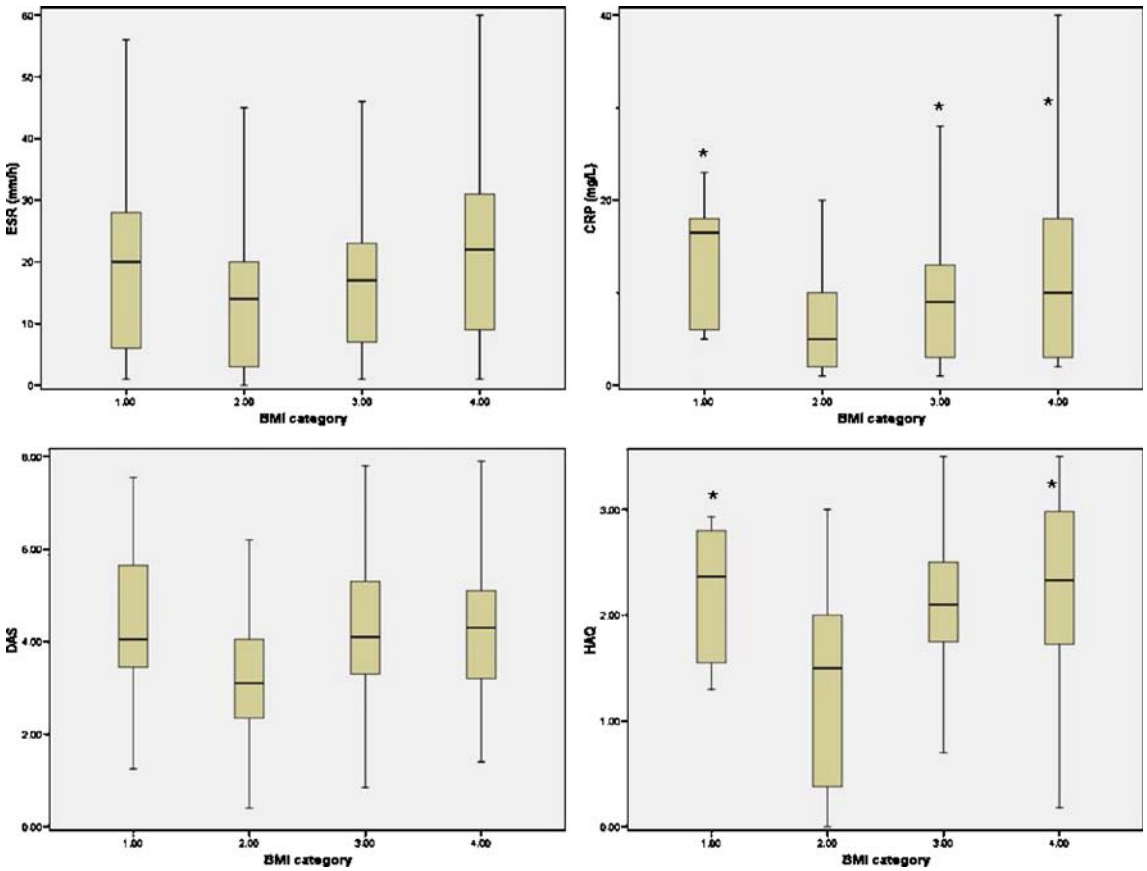

Fig. 1 Disease activity and physical function among BMI categories. *Asterisks*, significant difference compared to normal weight ($p<0.05$). *ESR* erythrocyte sedimentation rate; *CRP* C-reactive protein; *DAS* disease activity score 28; *HAQ* health assessment questionnaire

and HAQ; BF also associated with ESR, CRP and HAQ. These associations appear to be U-shaped, as both low and high BMI and BF associate with unfavourable disease activity and physical function. The differences between weight, BMI and BF in the observed associations might be explained by their varying ability to assess actual adiposity. Weight is a very generic measure that allows for large errors in the estimation of adiposity. BMI also has an inherent inability to distinguish between fat and fat-free body mass [18], which makes it a less accurate marker of adiposity than BF [19], particularly in conditions such as RA, which are characterised by significant alterations of body composition [6]. We were unable to find any associations of either weight, BMI or BF with the presence of erosions in radiographs of the hands and wrists. BMI was inversely associated with neck surgery, while BF associated positively with total knee replacement, but their overall influence on the total number of operated joints was not significant.

The present study has several potential limitations. The main one is its cross-sectional nature: the associations found are interesting and can serve for hypothesis generation, but they do not provide definitive evidence for causality or directionality, which can only be addressed in long-term prospective studies. The presence of erosions was assessed only qualitatively in radiographs of the hands and wrists: this does not allow quantitative analysis as all methods for quantification of erosions require X-rays from several different joints. Thus, patients with erosive disease were grouped together irrespective of the extent of erosive damage, and severity of joint damage could only be inferred by joint surgery. However, we have included these measures only as simple indications and we do not draw any of our major conclusions from them. Finally, body composition was assessed by bioelectrical impedance. This method has been validated [20–24] and is thought to be suitable for body composition studies in diverse populations [23–26], correlates well with the "gold standards" of dual-energy X-ray absorptiometry and hydrostatic weighing [24] and is widely used in RA research [6, 25, 27–30], but it has not actually been specifically validated in the RA population. On the other hand, even though a priori power calculations were not performed due to limited literature on the subject, retrospective power calculations, with HAQ as primary outcome, indicate that the size of the cohort gives a >0.99 power to our observations. Also the prospective collection of data in a standardised systematic manner minimised missing values and selection bias.

Overall, the associations of adiposity with RA disease characteristics found in this study are intriguing, in that

some of them are relatively easy to explain, while others seem counterintuitive. Pro-inflammatory cytokines, such as IL-1, IL-6 and TNFα, are clearly implicated in the pathogenesis and progression of RA [2, 31]. IL-6 stimulates liver production of CRP [32], a marker of inflammation and a measure of RA disease activity, and induces further release of IL-1 and TNF-α [33], which, amongst many other functions, can activate the transcription factor nuclear factor-kappa beta [34]; this is over-expressed in the inflamed synovium [35] and plays a central role in the initiation and progression of the chronic inflammation of RA [36]. The extent to which adipose tissue directly produces or indirectly induces the production of cytokines is still under intense investigation, but it is widely accepted that pro-inflammatory cytokine levels (such as IL-1, IL-6 and TNF-α) increase and anti-inflammatory cytokine levels (such as adiponectin, IL-1 receptor antagonist and IL-10) decrease with increasing adiposity [37]. We did not directly assess cytokine levels in this study, but this mechanism would be a good explanation for the higher disease activity (in terms of ESR, CRP or DAS28) and physical dysfunction (as reflected in the HAQ) observed in participants with increased BMI or BF.

This mechanism, however, does not explain the worse disease profile observed in underweight patients with very low BMI, which is in-line with evidence from other studies showing increased mortality levels among underweight RA patients [38]. In these patients, significantly reduced BMI is likely to be the result of highly active disease over many years [5] rather than vice versa, and it is interesting that low BMI appeared to associate with more neck surgery, which usually occurs in severe, uncontrolled and long-standing RA. The cross-sectional design of the present study and the relatively small number of underweight patients limit our ability to draw any definitive conclusions.

Counterintuitively, we were unable to demonstrate consistent associations between adiposity with the presence of erosions or joint surgery, despite the association of over-weight and obesity with higher ESR, CRP and HAQ. Within the aforementioned limitations of the study, a possible explanation for this is the previously reported protective effect of BMI against joint damage in early RA [8–10]. The protective effect seems to occur mainly in RF- and/or anti-CCP-positive patients [8, 10], to be present before the diagnosis of the disease, with over-weight or obese RA patients exhibiting less joint damage than their normal-weight counterparts at the time of diagnosis [8] and to continue during the first few years of the disease, with joint damage progressing less rapidly in obese than in normal-weight RA patients [8–10]. It is not entirely clear whether this is solely an effect of increased weight, for example, through increased mechanical loading stimulating bone synthesis [39], or also a reflection of joint damage at the time of first diagnosis. Our data suggest that although the protective effect of increased BMI may be less pronounced in established disease, it would still appear that obese patients with established RA do not exhibit increased levels of joint destruction, despite higher levels of systemic inflammation. A partial uncoupling between the acute-phase response and joint damage in RA has previously been suggested [40] and this may also be an explanation. In addition to this, it would be interesting to speculate an uncoupling between the effects of body weight (mainly reflected in weight) and adiposity (mainly reflected in BF): it is possible that the protective effects of body weight (i.e. increased mechanical loading of the bones) continue throughout the disease, whereas the deleterious effects of adiposity (i.e. increased inflammatory load) only "kick in" later, once a critical amount of fat has accumulated through the body composition changes occurring in RA.

In conclusion and within the limitations of this cross-sectional study, in established RA, both reduced and increased adiposity seem to be related to greater disease activity and physical dysfunction but not to more joint damage. These observations are independent from several potential confounders including RF and anti-CCP positivity. Further longitudinal studies are required to address this apparent dissociation.

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Disclosures None.

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Associations of obesity with modifiable risk factors for the development of cardiovascular disease in patients with rheumatoid arthritis

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ABSTRACT

Objectives: To assess the association of body mass index (BMI) with modifiable cardiovascular disease (CVD) risk factors in patients with rheumatoid arthritis (RA).

Methods: BMI, disease activity, selected CVD risk factors and CVD medication were assessed in 378 (276 women) patients with RA. Patients exceeding accepted thresholds in ⩾3 CVD risk factors were classified as having the metabolic syndrome (MetS).

Results: BMI independently associated with hypertension (OR = 1.28 (95% CI = 1.22 to 1.34); p = 0.001), high-density lipoprotein (OR = 1.10 (95% CI = 1.06 to 1.15); p = 0.025), insulin resistance (OR = 1.13 (95% CI = 1.08 to 1.18); p = 0.000) and MetS (OR = 1.15 (95% CI = 1.08 to 1.21); p = 0.000). In multivariable analyses, BMI had the strongest associations with CVD risk factors ($F_{1–354}$ = 8.663, p = 0.000), and this was followed by lipid-lowering treatment ($F_{1–354}$ = 7.651, p = 0.000), age ($F_{1–354}$ = 7.541, p = 0.000), antihypertensive treatment ($F_{1–354}$ = 4.997, p = 0.000) and gender ($F_{1–354}$ = 4.707, p = 0.000). Prevalence of hypertension (p = 0.004), insulin resistance (p = 0.005) and MetS (p = 0.000) was significantly different between patients with RA who were normal, overweight and obese, and BMI differed significantly according to the number of risk factors present (p = 0.000).

Conclusions: Increasing BMI associates with increased CVD risk independently of many confounders. RA-specific BMI cut-off points better identify patients with RA at increased CVD risk. Weight-loss regimens should be developed and applied in order to reduce CVD in patients with RA.

Rheumatoid arthritis (RA) associates with increased risk for cardiovascular disease (CVD).[1] This is most likely a combination of genetic predisposition,[2] modifiable CVD risk factors and the inflammatory burden of the disease.[3] Patients with RA have a significantly higher body fat content compared with healthy individuals of the same height and weight.[4] This led to the development of RA-specific body mass index (BMI) thresholds for overweight and obesity that better identify patients with RA with increased body fat,[4] and possibly CVD risk.

In the general population, obesity is a major contributor to dyslipidaemia, hypertension and insulin resistance[5] and the underlying cause of the metabolic syndrome (MetS).[5] The associations between obesity and CVD risk factors or the MetS in RA have not been extensively investigated. Obesity in this population is usually considered a confounder, against which data should be standardised, but not as the possible underlying cause for several CVD risk factors. The aim of this study was to quantify the associations of BMI with classical CVD risk factors in a large sample of patients with RA.

METHODS

Participants

The study had ethical approval and all volunteers provided informed consent. A total of 400 (289 women) consecutive patients with RA were assessed; of these, none had uncontrolled thyroid disease, but 22 were excluded due to cancer. The analyses from the remaining 378 (276 women) patients are reported: their characteristics appear in table 1.

Assessments

Standing height, weight, BMI (kg/m^2) and waist circumference were measured. CVD risk factors (blood pressure (BP), lipids) were assessed, smoking status noted and the Framingham 10-year CVD event probability was calculated. The Homeostasis Model Assessment of insulin resistance (HOMA) and the Quantitative Insulin sensitivity Check Index (QUICKI),were used to determine insulin resistance (IR). The NCEP ATP III criteria[5] were used to identify patients with the MetS. Erythrocyte sedimentation rate, C-reactive protein, disease activity score (using 28 joint counts) (DAS28) and the Stanford Health Assessment Questionnaire (HAQ) were also assessed.

Data analysis

The Statistical Package for Social Sciences version 15.0 was used (SPSS Inc. Chicago, Illinois, USA). Dispersion of the variables was assessed using the Kolmogorov–Smirnov test. Spearman's correlations explored the associations of CVD risk factors and 10-year CVD event probability with BMI. Results were standardised for gender, age, smoking status, RA characteristics and CVD medication using univariable analyses. Multivariable analyses were used to asses the overall association of each of the possible confounders with the CVD risk factors and the 10-year CVD event probability.

Following grouping according to RA-specific BMI[4] in those who were underweight, normal weight, overweight and obese, analysis of variance (ANOVA) was used to assess differences in BP, lipids and IR between groups. Analysis of co-variance

Table 1 Demographic and disease characteristics of volunteers (median (interquartile range))

	Male	Female
N	102	276
Age	63.5 (13.6)	63 (14.8)
Height	173.0 (9.8)	160.0 (8.0)
Weight	83.3 (19.7)	70.0 (19.9)
BMI	27.6 (5.5)	26.7 (7.2)
DAS28	4.0 (1.8)	4.2 (1.9)
HAQ	1.2 (1.8)	1.6 (1.5)
ESR	19.0 (30.0)	21.0 (28.0)
CRP	10.0 (15.0)	8.0 (13.0)
Disease duration	9.0 (15.0)	10.0 (15.0)

BMI, body mass index; CRP, C-reactive protein; DAS28, disease activity score (using 28 joint counts); ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire.

(ANCOVA) was then used to standardise for the same possible confounders as above. χ^2 analyses were used to identify differences in the prevalence of each risk factor or the MetS between BMI groups. Binary logistic models were used to test the independence of these associations from the same possible confounders.

Finally, patients were grouped according to the total number of risk factors they had. ANOVA was used to assess differences in BMI among these latter groups and ANCOVA to adjust for the same possible confounders. Data are reported as median (interquartile range). Statistical significance was set to $p<0.05$.

RESULTS

Body mass index and cardiovascular disease risk factors

BMI correlated significantly with systolic BP ($r = 0.240$, $p = 0.000$), high-density lipoprotein ($r = -183$, $p = 0.000$), HOMA ($r = 0.302$, $p = 0.000$) and QUICKI ($r = -0.300$, $p = 0.000$). BMI also correlated with erythrocyte sedimentation rate ($r = 0.128$, $p = 0.011$), C-reactive protein ($r = 0.155$, $p = 0.002$) and HAQ ($r = 0.133$, $p = 0.009$); therefore, results were standardised for these parameters as well as for gender, age, smoking and CVD medication. BMI retained its association with systolic BP ($F_{1–354} = 23{,}372$, $p = 0.000$), high-density lipoprotein ($F_{1–354} = 10.439$, $p = 0.001$), HOMA ($F_{1–354} = 11.311$, $p = 0.001$) and QUICKI ($F_{1–354} = 34.678$, $p = 0.000$) and also associated with diastolic BP ($F_{1–354} = 7{,}593$, $p = 0.006$), triglycerides ($F_{1–354} = 4.496$, $p = 0.035$) and 10-year CVD event probability ($F_{1–354} = 5.857$, $p = 0.016$). Different multivariate models, using BP, lipids and IR as dependent variables, gender and smoking status as factors and BMI, age, RA characteristics and CVD medication as covariates indicated that the variance observed in all CVD risk factors was more closely associated with BMI ($F_{1–354} = 8.663$, $p = 0.000$), followed by lipid-lowering treatment ($F_{1–354} = 7.651$, $p = 0.000$), age ($F_{1–354} = 7.541$, $p = 0.000$), antihypertensive treatment ($F_{1–354} = 4.997$, $p = 0.000$) and male gender ($F_{1–354} = 4.707$, $p = 0.000$).

ANCOVA, with corrections for gender, age, smoking, RA characteristics and usage of CVD medication, showed significant differences between BMI subgroups (underweight, normal weight, overweight and obese) for systolic BP ($F_{1–354} = 14.707$, $p = 0.000$), diastolic BP ($F_{1–354} = 6.457$, $p = 0.011$), triglycerides ($F_{1–354} = 4.700$, $p = 0.031$), high-density lipoprotein ($F_{1–354} = 7.545$, $p = 006$), HOMA ($F_{1–354} = 9.720$, $p = 0.002$), QUICKI ($F_{1–354} = 30.332$, $p = 0.000$) and 10-year CVD event probability ($F_{1–354} = 3.981$, $p = 0.046$).

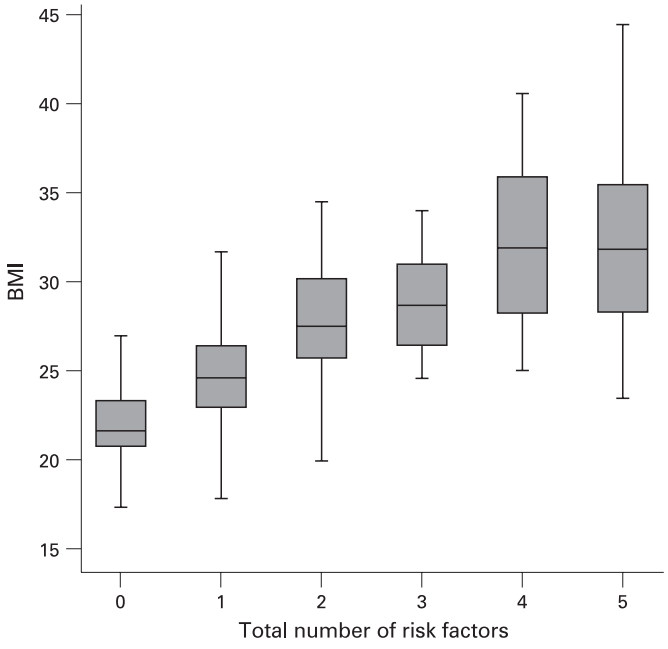

Figure 1 Mean (95% confidence interval of the mean) for body mass index (BMI) of participants according to risk factor grouping. Differences between groups in BMI are significant ($p = 0.000$). Risk factors include: hypertension, high triglycerides, low high-density-lipoprotein, insulin resistance and waist circumference.

Body mass index subgroups and the prevalence of cardiovascular disease risk factors

Cross-tabulation of BMI subgroups with presence or absence of each risk factor or the MetS demonstrated significant differences between groups for the prevalence of hypertension ($p = 0.004$), insulin resistance ($p = 0.005$) and the MetS ($p = 0.000$) (fig 1). The binary logistic models indicated that BMI associated with hypertension (OR = 1.28, 95% CI = 1.22 to 1.34; $p = 0.001$), high-density lipoprotein (OR = 1.10, 95% CI: 1.06 to 1.15; $p = 0.025$), IR (OR = 1.13, 95% CI = 1.08 to 1.18; $p = 0.000$) and the MetS (OR = 1.15, 95% CI: 1.08 to 1.21; $p = 0.000$) independently of confounding factors.

Following grouping for the total number of risk factors present, ANOVA showed significant differences in BMI between groups ($p = 0.000$; table 1), while ANCOVA revealed that this association was independent of gender, age, smoking, RA characteristics and use of CVD medication ($p = 0.000$).

DISCUSSION

These results suggest an almost linear relationship between BMI and CVD risk in this patient group, with the risk profile worsening as BMI increases, in a pattern similar to that described in the general population.[5] These associations were independent of multiple confounders, and if anything, they became stronger following inclusion of CVD medication in the models. It must be emphasised that these are all cross-sectional associations, and they do not provide definitive evidence for causality or directionality: longitudinal studies are required for this.

There is no reason to suggest that the mechanisms by which obesity increases CVD risk in RA are different from those in the general population. Excess adipose tissue releases non-esterified fatty acids in the circulation, which overload the liver and

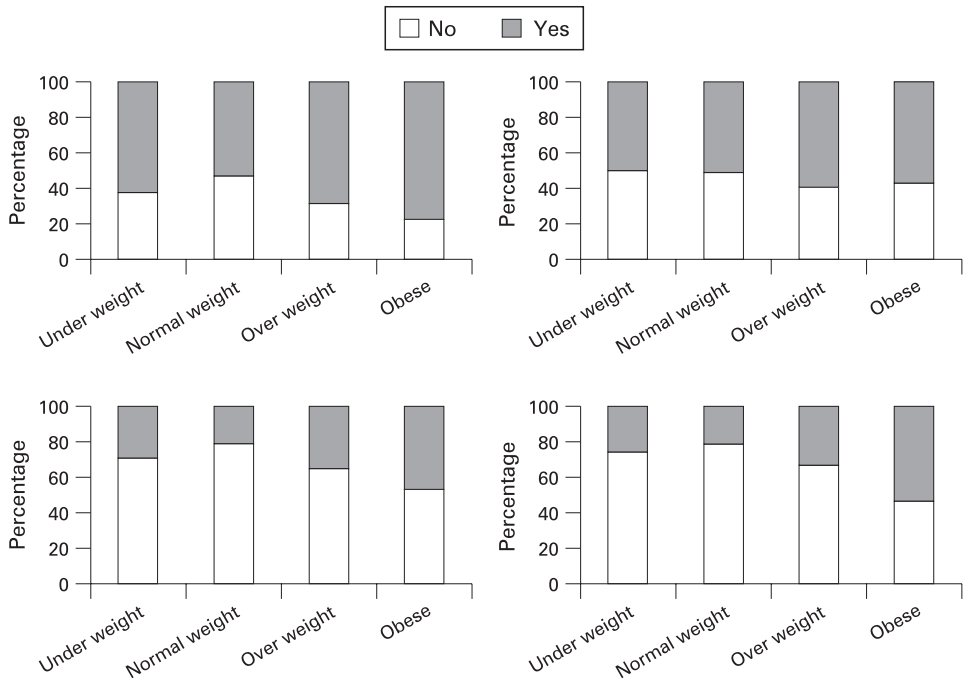

Figure 2 Prevalence of individual risk factors and the metabolic syndrome for each body mass index group. Significant differences between body mass index groups were found for the prevalence of hypertension ($p = 0.004$), insulin resistance ($p = 0.005$) and the metabolic syndrome ($p = 0.000$).

muscles with lipids and increase lipolysis, while reducing glucose utilisation. Circulating glucose stimulates insulin production, leading to insulin resistance.[6] Endothelial function is often impaired causing arterial stiffness and hypertension.[7] Obesity may also increase CVD risk by reducing adiponectin, activating the rennin–angiotensin–aldosterone system, and increasing sympathetic activity and renal sodium reabsorption.[6] However, as we did not measure any of these parameters we can only postulate about their contribution to our observations.

Recent studies in RA have shown no relation[8] or even a "paradoxical" protective effect of obesity against CVD,[9] although no potential mechanisms were described. In our study, lipid-lowering and antihypertensive drugs strongly associated with CVD risk factors; their inclusion in the models strengthened the association of BMI with all risk factors assessed. Such drugs, known to improve CVD risk and reduce mortality, are more frequently prescribed in obese than in non-obese individuals.[10] Thus their inclusion in the analyses of studies investigating CVD risk and outcome in RA is of paramount importance.

In the present study, neither disease characteristics nor smoking affected the associations of BMI with CVD risk. This finding is similar to our previous observations indicating that alterations in body composition of patients with RA occur in the early years of the disease[4] or even prior to it. This could be the case for some CVD risk factors as "the risk of coronary heart disease in RA patients precedes the ACR criteria-based diagnosis of RA".[11] Similarly, smoking appears to confer less CVD risk in RA than in the general population.[3] Most likely, this is the result of the smoking-induced weight-loss we recently described in RA,[12] which may counteract the known negative effects of smoking on risk factors. However, disease characteristics and smoking were treated solely as possible confounders, thus their direct associations with CVD risk in RA cannot be assessed in this study.

An important finding of the present study is the BMI level at which CVD risk increases. Patients with one risk factor had a median BMI of <25 kg/m^2, whereas those with MetS <30 kg/m^2 and by applying general BMI thresholds would be classified as normal weight and overweight respectively; however, based on RA-specific BMI thresholds they would be classified as overweight or obese. This could be important in routine clinical practice, where such classifications may be used to target patients at increased risk for screening, early identification and management of risk factors.

In the general population, weight loss can reverse the adverse effects of obesity. However, in a population with significant muscle wasting, such as RA, the type of weight-loss intervention has to be carefully considered. Among existing weight-loss regimens, exercise and especially resistance training, is the only one proven to increase muscle mass in the general population[13] and may be applied in patients with RA without aggravating their disease.[14] Moreover, exercise is known to further reduce CVD risk irrespective of weight loss.[15] Research focusing on weight-loss interventions and their effects on CVD in RA are necessary.

Within its limitations, this study shows that increasing BMI in patients with RA associates with increased CVD risk. The use of RA-specific BMI thresholds better identifies patients with RA at increased CVD risk. Weight-loss regimens specific for patients with RA need to be developed and evaluated.

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Competing interests: None.

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Research article

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Cigarette smoking associates with body weight and muscle mass of patients with rheumatoid arthritis: a cross-sectional, observational study

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Abstract

Introduction Rheumatoid arthritis (RA) is associated with altered metabolism leading to muscle wasting. In the general population, cigarette smoking is known to affect body composition by reducing fat and inhibiting muscle synthesis. Even though smoking has been implicated in the pathophysiology and progression of RA, its possible effects on body composition of such patients have not been studied. This cross-sectional study aimed to identify potential associations of smoking with body weight and composition of RA patients.

Methods A total of 392 patients (290 females) with RA were assessed for body mass index (BMI), body fat (BF), fat-free mass (FFM), and waist circumference. Erythrocyte sedimentation rate, C-reactive protein, Disease Activity Score-28, and Health Assessment Questionnaire score were used to assess disease activity and severity. Smoking habit (current smoker, ex-smoker, or never-smoker) and intensity (pack-years) were also noted.

Results Current smokers had a significantly lower BMI compared with ex-smokers (mean difference: male -2.6, 95% confidence interval [CI]: -3.5 to -1.7; female: -2.6, 95% CI: -4.8 to -0.5) and never-smokers (mean difference: male -1.8, 95% CI: -3 to -0.6; female: -1.4, 95% CI: -2.4 to -0.4). Similarly, the BF of current smokers was lower compared with that of ex-smokers (mean difference: male: -4.3, 95% CI: -7.5 to -1.2; female: -3.4, 95% CI: -6.4 to -0.4) and never-smokers (mean difference: male: -3.3, 95% CI: -6.3 to -0.4; female: -2.1, 95% CI: -4 to -0.2). FFM did not differ between groups. Finally, current smokers had a significantly smaller waist circumference compared with ex-smokers only (mean difference: male: -6.2, 95% CI: -10.4 to -1.9; female: -7.8, 95% CI: -13.5 to -2.1). Following adjustments for age, disease duration, and HAQ score, smoking remained a significant predictor for BMI ($P < 0.001$), BF ($P < 0.05$), and waist circumference ($P < 0.05$). Pack-years were inversely correlated with BF ($r = -0.46$; $P < 0.001$), and heavy smokers exhibited a significantly lower FFM ($P < 0.05$) compared with all other participants.

Conclusion Within the limitations of a cross-sectional study, it appears that cigarette smoking associates with reduced BMI and BF in patients with RA and heavy smoking associates with lower muscle mass. Smoking cessation appears to associate with increased BMI, BF, and waist circumference in these patients. These results should be confirmed in prospective studies. Given the numerous adverse effects of smoking on general health and RA, patients should be actively advised against it. However, smoking cessation regimes in RA may need to include more general lifestyle counselling, particularly about weight control.

ANCOVA = analysis of covariance; ANOVA = analysis of variance; BF = body fat; BMI = body mass index; CI = confidence interval; CRP = C-reactive protein; DAS28 = Disease Activity Score-28; ESR = erythrocyte sedimentation rate; FFM = fat-free mass; HAQ = Health Assessment Questionnaire; RA = rheumatoid arthritis; REE = resting energy expenditure.

Introduction

Rheumatoid arthritis (RA), the commonest inflammatory arthritis, is associated with altered metabolism [1]. Compared with healthy controls, RA patients exhibit elevated resting energy expenditure (REE) and enhanced muscle catabolism [2]. Such changes may lead to rheumatoid cachexia (that is, involuntary loss of fat-free mass [FFM] with a proportional increase of body fat [BF]) in the presence of stable body weight [3,4]. Body composition changes, particularly BF increase, may remain largely undetected by traditional assessments such as the body mass index (BMI) [5]. Increased BF, together with reduced levels of physical activity due to joint inflammation and damage [3,6], is associated with several comorbidities, including cardiovascular disease [7,8] as well as increased mortality [3].

Cigarette smoking is an important risk factor for several diseases [9]. It is also known to decrease body weight in healthy individuals by reducing appetite and increasing REE [10]. In contrast, smoking cessation may associate with significant weight increase, which constitutes a major deterrent to smoking control [11].

We have recently demonstrated that smoking further increases REE in RA [12] and this could potentially augment rheumatoid cachexia in these patients. Given the RA-related alterations in body composition and the comorbidity associated with them, the examination of potential contributors to muscle wasting, such as smoking, is important. The aim of this cross-sectional study was to detect potential associations between smoking and body weight, body composition, and rheumatoid cachexia in RA patients.

Materials and methods

Participants

Consecutive patients attending routine rheumatology clinics at the Dudley Group of Hospitals NHS Trust, UK, were invited to participate. All applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. The study had local research ethics committee and research and development directorate approvals, and all volunteers provided informed consent. A total of 400 volunteers (108 males and 292 females) with RA (1987 revised American College of Rheumatology criteria [13]) were assessed. Of them, 8 (6 males) were excluded from the analyses due to missing data for body composition. Data from the remaining 392 (median age: 63.1 [55.5 to 69.6] years; median disease duration: 10 [4 to 18] years) were analysed.

Assessments

All volunteers were subjected to the same data collection procedures overseen by the same trained investigators. Standing height was measured to the nearest 0.5 cm on a Seca 214 Road Rod portable stadiometer (Seca gmbh & co. kg., Hamburg, Germany). Body weight and composition (that is, BF and FFM) were assessed using a Tanita BC- 418 MA Segmental Body Composition Analyzer (Tanita Corporation, Tokyo, Japan). After initial manual entry of their demographic details, participants stood barefooted on the analyzer and held the handgrips provided until the apparatus printed the results. BMI was calculated on the basis of measured height and weight in kilograms per square metre. Waist circumference was also measured. Contemporary disease activity was assessed by the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and the Disease Activity Score-28 (DAS28) [14]. The Anglicised version of the 40-item Stanford Health Assessment Questionnaire (HAQ) [15] was used to measure physical dysfunction as a proxy of disease severity. Patients' self-reported smoking status and intensity (that is, pack-years) were noted.

Data management and analyses

Data were analysed using the Statistical Package for Social Sciences version 15.0 (SPSS Inc., Chicago, IL, USA). A preliminary evaluation of the variables using a Kolmogorov-Smirnov test of normality revealed that none of them required transformation to reach normality. Mean ± standard deviation was calculated for all variables. Differences in BMI, BF, and FFM between smoking groups are presented as mean differences with 95% confidence intervals (CIs).

According to their smoking status, patients were grouped into never-smokers, current smokers, and ex-smokers. Analysis of variance (ANOVA) assessed differences in demographic characteristics, BMI, and body composition between groups for each gender. Analysis of covariance (ANCOVA) was employed to determine whether the differences observed were attributed to smoking status or other confounding factors (for example, gender, age, and disease characteristics).

In the current smoker and ex-smoker groups, further associations between pack-years with BMI and body composition were examined. Thereafter, patients in these groups were divided into quartiles according to pack-years. ANOVA was employed to assess differences in the measured variables between these subgroups. ANCOVA was used to correct for any confounding factors.

Thereafter, patients were grouped according to (a) RA-specific BMI [5] and (b) gender-specific BF [16] thresholds into underweight, normal weight, overweight, and obese. Subsequently, they were grouped based on gender-specific cut-off

points for waist circumference [17] into low or high risk and for FFM into low or normal FFM groups [18]. Chi-square analyses were employed to assess differences between smoking groups in the prevalence of overweight, obesity, high risk, and low FFM. For all tests, the level of significance was set at a *P* value of less than 0.05.

Results

Table 1 illustrates means ± standard deviations and the ANOVA results for all studied parameters. Current smokers had a significantly lower BMI than ex-smokers (mean difference: male -2.6, 95% CI: -3.5 to -1.7; female: -2.6, 95% CI: -4.8 to -0.5) and never-smokers (mean difference: male -1.8, 95% CI: -3 to -0.6; female: -1.4, 95% CI: -2.4 to -0.4). Current smokers also had a significantly lower BF compared with ex-smokers (mean difference: male: -4.3, 95% CI: -7.5 to -1.2; female: -3.4, 95% CI: -6.4 to -0.4) and never-smokers (mean difference: male: -3.3, 95% CI: -6.3 to -0.4; female: -2.1, 95% CI: -4 to -0.2). FFM did not differ between these groups (mean difference: current smokers versus ex-smokers, male: -4.6, 95% CI: -10.7 to 1.6; female: -1.2; 95% CI: -3.8 to 1.4; current smokers versus never-smokers, male: -2.7, 95% CI: -9.2 to 3.9; female: 0.1, 95% CI: -2.4 to 2.4). Current smokers had a significantly smaller waist circumference than ex-smokers (mean difference: male: -6.2, 95% CI: -10.4 to -1.9; female: -7.8, 95% CI: -13.5 to -2.1) but not never-smokers (mean difference: male: -2.9, 95% CI: -10.6 to 4.9; female: -3.9, 95% CI: -9.2 to 1.5). Also, ex-smokers had a larger waist circumference than never-smokers but the difference was significant for males only (mean difference: male: 3.3, 95% CI: 0.4 to 6.3; female: 3.9, 95% CI: -0.4 to 8.1).

In ANCOVA with gender and smoking as factors and age, DAS28, HAQ score, and disease duration as covariates, smoking was a significant and independent predictor for BMI ($F_{2,387} = 8$; $P < 0.001$), BF ($F_{2,387} = 4.4$; $P < 0.05$), and waist circumference ($F_{2,387} = 7.9$; $P < 0.001$). Smoking also emerged as a significant predictor of FFM ($F_{2,387} = 5.1$; $P < 0.05$), but inclusion of BMI as a covariate eliminated the effect of smoking on FFM ($P > 0.05$).

There was a significant negative correlation between pack-years and BF ($r = -0.46$; $P < 0.001$) in the current smoker and the ex-smoker groups. This remained significant after adjustment for gender, age, DAS28, HAQ score, and disease duration ($F_{1,389} = 4.8$; $P < 0.05$). Following pack-year grouping into quartiles (pack-group), ANOVA did not reveal any differences for BMI or body composition among the current and ex-smoker pack-groups. However, an ANCOVA model with gender and pack-group as factors and age and weight as covariates (following stepwise elimination of ESR, CRP, DAS28, HAQ score, and disease duration) revealed a significant effect of pack-group on FFM ($F_{3,217} = 2.7$; $P < 0.05$), with heavy smokers exhibiting the lowest values. Mean (95% CI) values of this variable in the pack-year subgroups appear in Figure 1.

Following BMI and BF grouping, chi-square analyses showed significant differences ($P < 0.05$) in the prevalence of over-

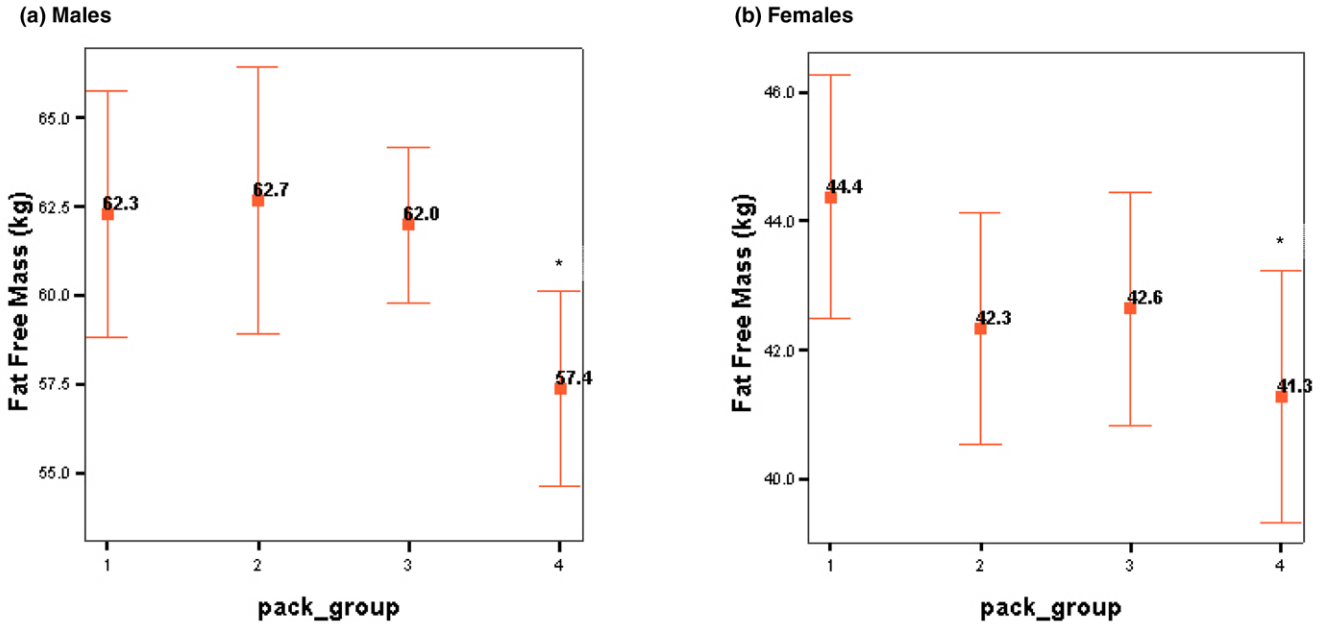
Table 1

Measured variables of participants classified as current smokers (CS), ex-smokers (XS), and never-smokers (NS)

Gender	Male (n = 102)			Female (n = 290)		
Smoking status	CS	XS	NS	CS	XS	NS
Number	20	50	32	49	97	144
Age, years	58.8 ± 8.1[a]	65.2 ± 9.9[b]	58.8 ± 15	57.4 ± 13.3[a]	64.1 ± 11.2[b]	60.7 ± 11.8
Height, cm	171.3 ± 7.1	174.3 ± 6.9	172.7 ± 7.7	160.9 ± 6.9	160.8 ± 6.8	159.5 ± 6.8
Weight, kg	76 ± 12.9[b, c]	85.8 ± 13.6	84.1 ± 14.8	67.5 ± 14.2[a]	74.8 ± 15.2	69.9 ± 13.6
Body mass index, kg/m^2	25.8 ± 3.3[b, c]	28.4 ± 3.8	27.6 ± 4.6	26.1 ± 5.5[a, b]	28.6 ± 5.4	27.5 ± 5
Body fat, percentage	24.5 ± 6.4[c, d]	28.8 ± 6.8	27.8 ± 5.6	35.9 ± 7[a, b]	39.2 ± 6.5	38.1 ± 6.7
Fat-free mass, kg	57.2 ± 9.4	61.7 ± 7.7	59.8 ± 10.3	42.5 ± 4.8	43.7 ± 6.1	42.5 ± 6.1
Waist circumference, cm	100 ± 7.9[c]	106.2 ± 10.8[b]	102.9 ± 9.3	90.8 ± 12.8[a]	98.6 ± 13	94.7 ± 12.7
ESR, mm/hour	26.5 ± 20.5	22.8 ± 21.3	20.7 ± 19.7	30.5 ± 26	34.3 ± 32.7[b]	25.5 ± 19.8
C-reactive protein, mg/L	13.3 ± 9.4	16.1 ± 20.4	16 ± 24.3	21.9 ± 23.2[b]	21.4 ± 32.7[b]	11.9 ± 12.5
DAS28	4 ± 0.9	4.1 ± 1.5	3.9 ± 1.6	4.5 ± 1.5	4.3 ± 1.5	4.1 ± 1.2
HAQ score	0.9 ± 0.8	1.4 ± 1	1.1 ± 0.9	1.5 ± 0.9	1.5 ± 0.9	1.5 ± 0.9
Disease duration, years	8.6 ± 7.8	11.9 ± 10.6	14.6 ± 12.7	11.4 ± 9.8	13.5 ± 10.8	13.5 ± 11.1

Values are presented as mean ± standard deviation. [a]Significant difference compared with XS ($P < 0.05$). [b]Significant difference compared with NS ($P < 0.05$). [c]Significant difference compared with XS ($P < 0.001$). [d]Significant difference compared with NS ($P < 0.001$). DAS28, Disease Activity Score-28; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire.

Figure 1



Fat-free mass for males **(a)** and females **(b)** according to pack-year grouping. Data are presented as means with 95% confidence intervals. Pack-year groups: 1, 1 to 9 pack-years; 2, 10 to 19 pack-years; 3, 20 to 34 pack-years; 4, greater than 35 pack-years. Asterisk indicates significant difference compared with group 1 ($P < 0.05$).

weight and obesity among smoking groups, with obesity being more prevalent in ex-smokers (50%) followed by never-smokers (39%) and current smokers (30%). Similarly, ex-smokers had a significantly ($P < 0.05$) higher prevalence of increased waist circumference (69%) compared with never-smokers (60%) and current smokers (49%). However, FFM did not differ between groups ($P > 0.05$) (Figure 2).

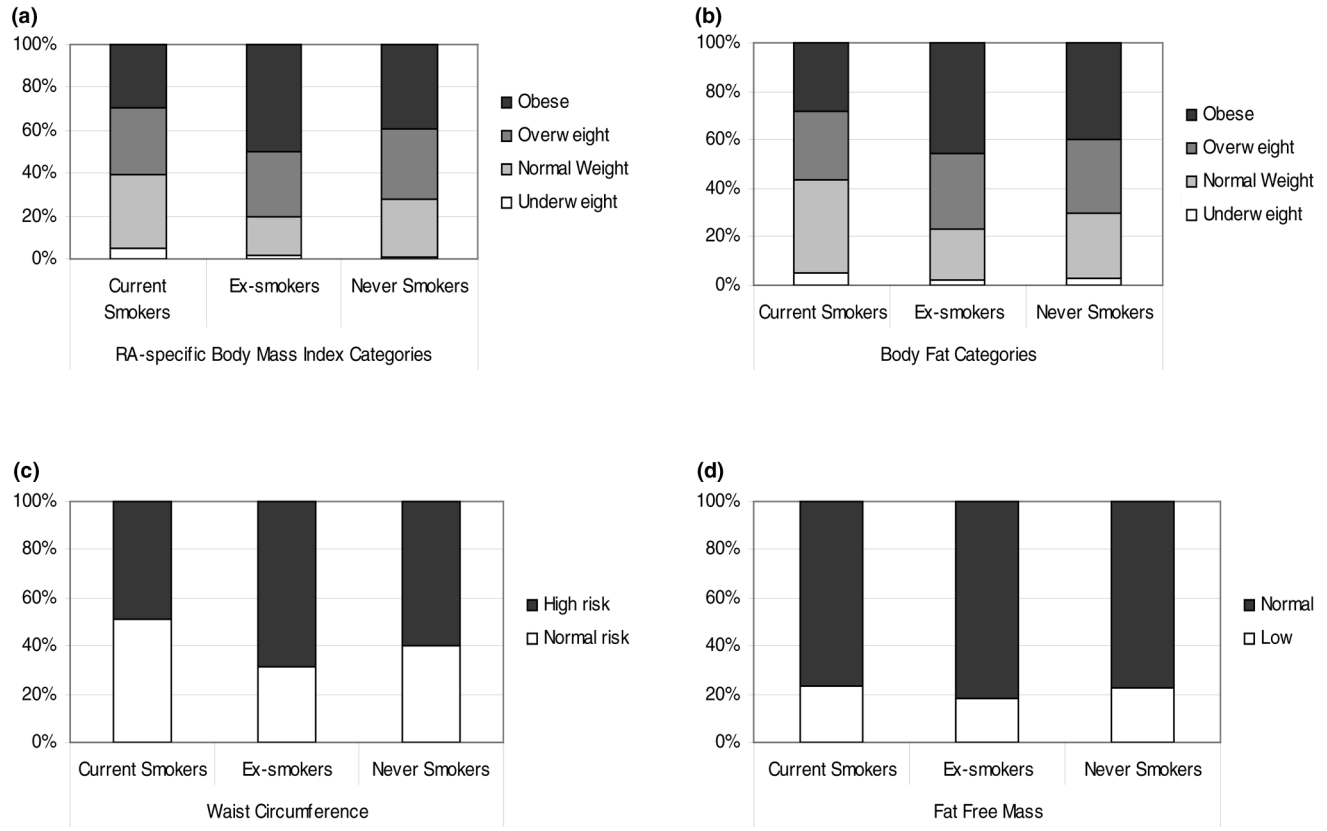
Discussion

To our knowledge, this is the first study to identify significant associations between smoking, body weight, and body composition of RA patients: current smokers had a significantly lower BMI and BF compared with never-smokers. Both BMI and BF were significantly increased in ex-smokers, whereas very heavy smoking appeared to associate with reduced FFM. The study has several potential limitations. These are all cross-sectional associations, and although they can serve for hypothesis generation, they do not provide definitive evidence for causality or directionality: longitudinal studies are required for this. In addition, body composition was assessed by bioelectrical impedance. This method has been validated [19-23] and is thought to be suitable for body composition studies in diverse populations [22-25], correlates well with the 'gold standards' of dual-energy x-ray absorptiometry and hydrostatic weighing [23], and is widely used in RA research [5,12,24,26,27], but it has not actually been specifically validated in the RA population. Finally, although self-report of smoking, especially smoking history, is generally reliable, both under- and over-reporting can occur [28]. This is unlikely to have influenced the primary findings of this study (that is, the differences between current, ex-, and non-smokers), while any misreporting in pack-years may have been smoothed by the large number of participants. It is difficult to assess any other selection bias: the prevalence of current, ex-, and non-smokers among the participants of this study was similar to that reported for local general population subjects of similar age [29], although it was different from an RA cohort established more than 10 years ago [30].

Our observations for BMI are consistent with those in the general population. Both male and female smokers tend to have decreased BMI compared with their non-smoking counterparts [10,31]. In contrast, significant BMI increases have been noted after smoking cessation [11]. Smokers have increased levels of leptin [32], which regulates food intake and fat deposition [33], and reduced hypothalamic neuropeptide Y [34], which regulates appetite [35]. Smoking-induced increases in the levels of epinephrine, norepinephrine, and thyroid hormones lead to increased energy expenditure at rest [36,37] and during light physical activity [38-40]. However, these effects are short-lived: after smoking cessation, leptin decreases to levels below those expected for non-smokers of similar weight [32] and resting energy expenditure (REE) returns to normal [41].

In patients with RA, smoking has been shown to elevate REE [12]; however, no data are available on other potential contributors to smoking-related weight loss or smoking cessation-

Figure 2



Prevalence of overweight and obesity, increased waist circumference, and low fat-free mass in smoking groups. **(a)** Prevalence of overweight and obesity based on rheumatoid arthritis (RA)-specific body mass index for current, ex-, and never-smokers. **(b)** Prevalence of overweight and obesity based on body fat for current, ex-, and never-smokers. **(c)** Prevalence of high risk based on waist circumference for current, ex-, and never-smokers. **(d)** Prevalence of low fat-free mass for current, ex-, and never-smokers. Chi-square analyses identified significant defences among smoking groups for prevalence of **(a)** overweight and obesity based on body mass index ($P < 0.05$), **(b)** overweight and obesity based on body fat ($P < 0.05$), and **(c)** increased waist circumference ($P < 0.05$). Prevalence of low fat-free mass did not differ between groups ($P > 0.05$).

related weight gain for this population. Although we did not assess energy intake and expenditure or related regulators (such as leptin), it is likely that the mechanisms behind the reduced body weight of current smokers and the increased body weight of ex-smokers with RA are similar to those described for the general population.

Interestingly, the lower BMI of current smokers in the present study seems to be due to decreased BF rather than FFM. A possible mechanism by which smoking may affect fat metabolism is through a reduction in neuropeptide Y. This molecule not only stimulates food intake, but also promotes white fat lipid storage and decreases brown fat thermogenesis [35], so its inhibition through smoking would be expected to have the opposite effects. Additionally, smoking results in decreased adipose tissue lipoprotein lipase activity [42], which diverts fat storage away from adipose tissue and toward utilization by muscle [43], possibly leading to the decreased BF of smokers [42,44]. In the present study, the inverse association between smoking and BF appeared to be dose-dependent: increasing pack-years associated with reducing BF levels. Smoking cessation is thought to result in a reversal of the mechanisms described above, leading to increases in BF [42] and, most importantly, abdominal fat [45]. Indeed, among these RA patients, ex-smokers seemed to be the most 'unhealthy' group in terms of body weight and composition as they exhibited the highest BMI, BF, and waist circumference values.

In predominantly healthy people who are from the general population and who do not have wasting muscle disease, smoking of any intensity has been implicated in muscle wasting [10] by impairing the process of muscle protein synthesis [46]. In contrast, in the present study, only very heavy smoking appeared to associate with a reduction in FFM. It is possible that the effect of smoking on muscle is of less significance than the muscle loss associated with RA itself, as part of rheumatoid cachexia. This hypothesis is supported by the finding that increased duration of smoking (that is, pack-years) associated with lower FFM in both current and ex-smokers, which suggests the existence of a threshold below which smoking does

not induce further muscle loss in RA patients. A longitudinal study of the impact of smoking intensity (and cessation) on the body composition of patients with RA may throw more light on the mechanistic basis of these observations.

Overall, this study suggests that, in RA, smoking associates with reduced body mass and fatness without inducing further muscle loss, except in very heavy smokers; in contrast, smoking cessation associates with increased body mass and fatness. This should not be interpreted as favouring what is a very unhealthy habit. Smoking cessation, even if it occurs in midlife, reduces most of the later risk of death from tobacco [47]. However, smoking cessation is known to result in body weight increase, and this may affect some people's decision to stop smoking [11,44,45]. Therefore, any smoking cessation regime should be underpinned by more generalised lifestyle counselling, including advice on exercise and weight management. This is emphasized by the fact that, based on recently described RA-specific BMI [5], BF [16], and waist circumference thresholds [48], ex-smokers have the highest prevalence of obesity – both total and abdominal. FFM did not differ between groups and the prevalence of low FFM was comparable to that expected in age- and gender-matched healthy individuals [18].

Conclusion

Within the limitations of this study, it is concluded that RA smokers have a lower BMI and BF than RA non-smokers, while heavy smokers also have a reduced FFM. A history of smoking cessation appears to associate with increases in BMI, BF, and waist circumference. Nevertheless, given the numerous adverse effects of smoking on health, smokers with RA should be actively advised against it, but smoking cessation programs should include wider lifestyle counselling for weight control, also focusing on increased physical activity and a healthy diet.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AS-K participated in patient recruitment, data collection and analysis, and the drafting of the manuscript. GSM participated in patient recruitment and in data collection and analysis. VFP and KMJD participated in patient recruitment, rheumatological clinical assessments, and application of diagnostic/classification criteria. AMN provided expert statistical advice and supervision and participated in the review of the manuscript. AZJ participated in the inception and development of protocol and in the review of the manuscript and served as PhD program supervisor. MK provided advice on protocol development and body composition assessments and participated in the review of the manuscript. YK participated in the inception and development of protocol and served as PhD program supervisor. GDK participated in the inception and development of protocol, patient recruitment, clinical assessments, and analytical approach, provided supervision in the drafting of the manuscript, and served as PhD program supervisor and study guarantor.

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