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BODY COMPOSITION AND BONE MINERAL DENSITY IN RHEUMATOID ARTHRITIS- INFLUENCE OF INFLAMMATION AND TREATMENT WITH GLUCOCORTICOIDS AND TNF-BLOCKING AGENTS

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory disease. Besides symptoms from the joints, changes in body composition and reduced bone mineral are common. Increased fat mass (FM) and loss of muscle mass contribute to increased morbidity and mortality. The aim of this thesis was to study the impact of inflammation and medical treatment on body composition and bone mineral density (BMD) in patients with RA. The work is based on two cross-sectional studies in established RA and two prospective, randomized studies in early RA, analysing the effects of glucocorticoids (GCs) and anti-TNF therapy, respectively. The patients were assessed clinically and by assays, such as markers of bone remodelling, insulin-like growth factor-1 (IGF-1), apolipoproteins, leptin and adiponectin. Dual X-ray energy absorptiometry (DXA) was performed and fat free mass index (FFMI, kg/m^2) and fat mass index (FMI, kg/m^2) were calculated.

Low fat free mass (FFM), below the 10^{th} percentile of a reference material, was frequent. The highest frequency, 50%, was found in RA inpatients and was associated with high disease activity, physical disability and low bioavailable IGF-1. The proportion of outpatients with established disease that had low FFM was 38% and in patients with early RA 19%. Between 34 and 45% of the patients had high FM, above the 90^{th} percentile of the reference population, and 80% had FM% corresponding to overweight or obesity. The frequency of osteoporosis was 26-28% in established RA and 9% in early RA. In established RA, patients with long-term GC therapy had higher FM than those without, whereas BMD in lumbar spine and femoral neck did not differ between patients treated with GC and those not. Further, there was no significant difference between the treatment groups in the markers of bone formation. In contrast, in the prospective study in early RA, GC treatment was associated with a rapid decrease in the marker of bone synthesis during the first 3 months in contrast to patients not treated with GC. Also the markers of bone resorption decreased and it seemed that GC treatment could counteract the negative impact of inflammation on bone tissue, as BMD in femoral neck was preserved after 2 years treatment. However, GC treatment could not prevent bone loss in spine in postmenopausal women, where BMD decreased significantly more than in those not treated with GC. Treatment with TNF antagonists was associated with a significant increase in FM after 2 years, an increase that was not found in patients treated with a combination of disease modifying anti-rheumatic drugs (DMARDs), despite similar reduction of disease activity. The increase of FM seemed thus to be a specific effect of anti-TNF blockade and was not associated with an atherogenic lipid profile. Further, levels of leptin and adiponectin increased, of which adiponectin normally is associated with improved insulin sensitivity and endothelial function. This may at least partially explain the reduced frequency of CVD found when disease activity is reduced in RA, also when anti-TNF therapy is used.

In conclusion, a large proportion of RA patients have changes in body composition, which contribute to increased morbidity and mortality. GC treatment is associated with increased FM and can counteract the negative impact of inflammation on bone but need special attention to postmenopausal women. Anti-TNF therapy also increases FM but at the same time increases adiponectin, which have favourable effects on CVD risk factors.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I. **Engvall IL**, Elkan AC, Tengstrand B, Cederholm T, Brismar K, Hafström I. Cachexia in rheumatoid arthritis is associated with inflammatory activity, physical disability and low bioavailable insulin-like growth factor. Scand J Rheumatology 2008;37:321-328

II. **Engvall IL**, Brismar K, Hafström I, Tengstrand B. Treatment with low-dose prednisolone is associated with altered body composition but not bone mineral density in rheumatoid arthritis patients- a controlled cross-sectional study. Submitted

III. **Engvall IL**, Svensson B, Tengstrand B, Brismar K, Hafström I for the BARFOT study group. Impact of low-dose prednisolone on bone synthesis and resorption in early rheumatoid arthritis: experiences from a two-year randomized study. Arthritis Res Ther 2008;10(6) R128

IV. **Engvall IL**, Tengstrand B, Brismar K, Hafström I. Anti-tumor necrosis factor therapy increases body fat mass in early rheumatoid arthritis independently of changes in disease activity and levels of leptin and adiponectin- a randomized study over two years. Submitted

CONTENT

LIST OF ABBREVIATIONS

ACR	American College of Rheumatology
AMPK	Adenosine monophosphate activated protein kinase
ANCOVA	Analysis of covariance
ApoA1	Apolipoprotein A1
ApoB	Apolipoprotein B
AUC	Area under the curve
BARFOT	Better Anti-Rheumatic FarmacOTherapy
BCM	Body cell mass
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
CI	Confidence interval
CRP	C-reactive protein
1CTP	C-terminal telopeptide of type I collagen
CTX-1	C-telopeptide crosslaps of type I collagen
CV	Coefficient of variation
CVD	Cardiovascular diseases
DAS28	Disease Activity Score of 28 joints
DMARD	Disease –modifying antirheumatic drug
DXA	Dual-energy X-ray Absorptiometry
ECLIA	Electrochemiluminescence immunoassay
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
FFA	Free fatty acid
FFM	Fat free mass
FFMI	Fat free mass index
FM	Fat mass
FMI	Fat mass index
GC	Glucocorticoid
HAQ	Health Assessment Questionnaire
HCQ	Hydroxychloroquine
HDL	High density lipoprotein
HOMA-IR	Homeostasis model assessment of insulin resistance
11HSD	11 Beta hydroxysteroid dehydrogenase
IGF-1	Insulin-like growth factor-1
IGFBP-1	Insulin-like growth factor binding protein-1
IL-1	Interleukin-1
IL-6	Interleukin-6
INF	Infliximab
IQR	Inter quartile range
IRS-1	Insulin receptor substrate-1
LBM	Lean body mass
LDL	Low density lipoprotein
MMP	Matrix metalloproteinases

MNA	Mini Nutritional Assessment
MTX	Methotrexate
NoP-group	No prednisolone treated group
NFκB	Nuclear factor kappa B
OPG	Osteoprotegerin
P-group	Prednisolone treated group
PI3K	Phosphatidyl inositol 3 kinase
P1NP	Procollagen type I N-terminal propeptide
PPARα	Peroxisome proliferator activated receptor alpha
RA	Rheumatoid arthritis
RANK	Receptor activator of nuclear factor kappa B
RANKL	Receptor activator of nuclear factor kappa B ligand
RF	Rheumatoid factor
RIA	Radioimmunoassay
SD	Standard deviation
SE	Shared epitope
SGA	Subjective Global Assessment
SSZ	Sulphasalazine
TG	Triglyceride
TNFα	Tumor necrosis factor alpha
UPS	Ubiquitin Proteasome System

1 BACKGROUND

Rheumatoid arthritis (RA) is a chronic inflammatory disease with a prevalence of 0.5-1.0% and it is 2-3 times more common in women. The age of disease onset peaks in the fifth decade (2). The disease primarily affects the synovial joints with swelling, pain and morning stiffness. With time cartilage damage and joint erosions contribute to joint destruction with subsequent physical impairment. Besides symptoms from the joints, systemic features such as fatigue and extra-articular involvement of other organs, i.e. the skin, lungs, eyes, nervous system, blood vessels or exocrine glands may occur. RA also affects body composition with loss of muscle mass in presence of stable or increased fat mass, a condition known as rheumatoid cachexia. With time peri-articular and generalized osteopenia may develop into osteoporosis with increased risk of fractures (44, 88, 171). Life expectancy is decreased in patients with RA. The most common cause of death in RA patients is cardiovascular diseases (CVD) (67, 182). The incidence of acute myocardial infarct is increased by 1.5-4 times that in the general population (40, 180) and the mortality following a CVD event is also increased (182).

1.1 RHEUMATOID ARTHRITIS

1.1.1 Etiology

The etiology of RA is unknown but it is suggested that a genetic susceptibility in combination with the influence of environmental factors are involved. The best known genetic association is to the human leukocyte antigen (HLA) class II region, HLA-DR and particular to those allelic forms that code for the so-called shared epitope (SE) (72). Several other genetic markers have also been identified (140). Smoking has been reported to be an environmental risk factor that increases the risk of developing rheumatoid factor (RF) positive RA (175). Smoking also increases the risk of developing anti-cyclic citrullinated peptide antibodies (ACPA) in individuals with the SE allele (97). Gender and hormonal factors are also important with higher prevalence in women than in men, a difference that is less obvious after menopause (175).

1.1.2 Pathogenesis

The inflammation usually starts in the synovial joints and more specifically in the synovium. Different antigens activate macrophage-like and fibroblast-like synoviocytes, resulting in synovium hyperplasia followed by infiltration by macrophages and lymphocytes. This inflamed tissue, called pannus, extends over and invades the cartilage and bone. The cells in the pannus produce excessive amount of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukin-1 beta (IL-1β), IL-6 and receptor activator of nuclear factor κB ligand (RANKL). Neutrophils from the circulation are attracted to the synovial fluid by chemo attractants. Fibroblasts, macrophages and neutrophils produce proteolytic enzymes and oxygen metabolites that contribute to matrix degradation. Besides resulting cartilage- and bone degradation, pro-inflammatory cytokines are also found in the circulation leading to systemic symptoms and manifestations.

Earlier studies have indicated TNFα being at the top of a cascade of pro-inflammatory cytokines and that blockade of TNFα would suppress the production of other inflammatory mediators. Today we use biologic agents targeting TNFα, IL-1 and IL-6

in the treatment of RA. Primer on the Rheumatic Diseases (Klippel et al 2001), section 9A, serves as a general reference (68).

1.1.3 Diagnosis and classification

As no single diagnostic symptom, sign or test exists, the diagnosis is often based upon the classification criteria adapted by the American College of Rheumatology (ACR) in 1987 (9), table 1. The criteria were originally created for research rather than diagnostic purposes and were developed on patients with established disease. The sensitivity of the ACR criteria is low in early disease (162).

Table 1. The 1987 American College of Rheumatology classification criteria for RA

Criterion	Definition
1. Morning stiffness	Morning stiffness in and around the joints, lasting at least 1 hour before maximal improvement.
2. Arthritis of three or more joint areas	At least three joint areas simultaneously have had soft tissue swelling or fluid (not bony overgrowth) observed by a physician. The 14 possible areas are the right or left proximal interphalangeal (PIP), metacarpophalangeal (MCP), wrist, elbow, knee, ankle and metatarsophalangeal (MTP) joints.
3. Arthritis of hand joints	At least one area swollen in a wrist, MCP or PIP joint.
4. Symmetric arthritis	Simultaneous involvement of the same joint areas (as defined in 2) on both sides of the body (bilateral involvement of PIP, MCP or MTP joints is acceptable without absolute symmetry).
5. Rheumatoid nodules	Subcutaneous nodules over bony prominences, extensor surfaces or in juxta-articular regions, observed by a physician.
6. Rheumatoid factor (RF)	Detected by a method positive in less than 5% of normal controls.
7. Radiographic changes	Radiographic changes typical of RA on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify).
Patients fulfilling at least 4 of these 7 criteria are classified as having RA. Criteria 1 through 4 must have been present for at least 6 weeks. Patients fulfilling two clinical diagnoses are not excluded.	

1.2 BODY COMPOSITION AND BONE MINERAL DENSITY

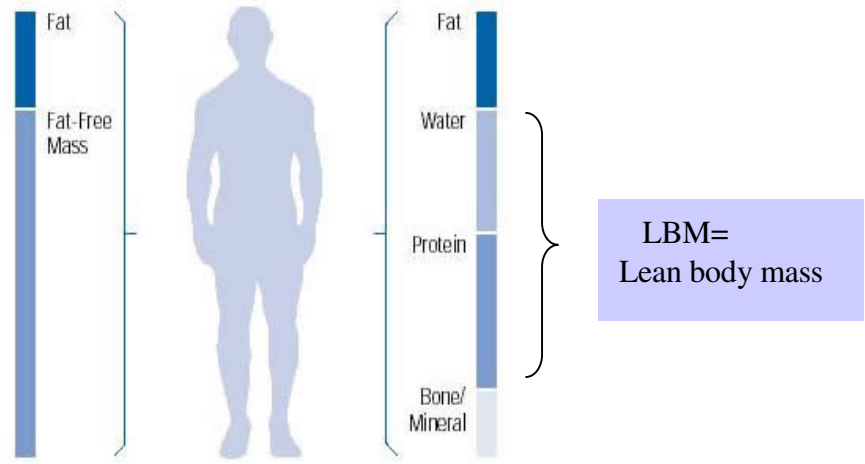
Traditionally body mass index (BMI) has been used to classify individuals as under- and overweight. It is calculated by dividing body weight by the square of height (kg/m^2). A weakness with BMI is that the actual composition of the body is not taken into account. An increased body weight may depend on excess adipose tissue, or alternatively, on muscle hypertrophy, while low BMI may be due to a loss of adipose tissue and/or due to low muscle mass. Many individuals with low BMI are not actually lean in terms of body fat (82) and individuals with normal BMI may have unrecognized low muscle mass.

Both low fat free mass (FFM), see below, and high fat mass (FM) have been shown to be associated with morbidity and mortality (82, 107), facts that point out the importance of determining body composition, instead of relying on only BMI. The known J-shaped association between mortality and BMI may be a result of compound risk functions of FFM and FM. Heitmann et al have shown that FFM has a negative linear association with mortality whereas FM has a positive linear association with mortality (82).

1.2.1 Body composition measurements

The body can be divided into different compartments. In the simplest way it can be divided into 2 compartments; FM and FFM. The FFM can be further divided into bone mineral, protein and water. The sum of body water and protein is called lean body mass (LBM), including skeletal muscle mass, viscera and cells of the immune system, figure 1.

Figure 1. Different compartments of the body



There are several different methods to measure body composition as reviewed by Summers (173). Sometimes a combination of different techniques is used.

Anthropometric methods include BMI, triceps skin-fold and arm circumference. BMI is discussed above. The other two methods are simple bedside techniques for measuring fat and muscle mass, respectively, but are of limited accuracy and reproducibility. Waist circumference is a simple measurement and is used as a marker for abdominal FM. It is strongly correlated with type 2 diabetes and CVD. It is also included as one of the diagnostic criteria for the metabolic syndrome (129).

Bioelectric impedance analysis (BIA) is based on the principle that fat and lean mass have different electrical conductivity. It is a simple, quick and inexpensive method to measure body composition. We have recently validated this method against dual X-ray absorptiometry (DXA) in patients with RA (51) but BIA is not used in any of the papers included in this thesis.

Dual- energy X-ray Absorptiometry (DXA) is used for many clinical and research purposes and was developed 20 years ago to measure bone mineral. It gives measurements of three body compartments; FM, LBM and bone mineral content (BMC) as well as bone mineral density (BMD). DXA is based on the fact that an X-

ray beam passing through the body is attenuated to a different extent depending on the different substances it passes through. Using a dual beam with two distinct energy peaks, one absorbed mainly by soft tissue and the other by bone, the machine can divide the body into two compartments. The soft tissue compartment is then analysed to separate fat mass from LBM. The use of DXA is suggested to be a fairly exact method to measure FM and FFM, it is often used as a reference method (105) and is considered to be sensitive to changes in body composition (66, 141). The radiation dose is low, close to background radiation levels. In this thesis DXA is used for all measurements of body composition and BMD.

Imaging techniques like computer tomography (CT) and magnetic resonance imaging (MRI) provide good measurements of skeletal muscle mass and fat. They also distinguish visceral fat tissue from subcutaneous fat tissue. The disadvantages are that they are expensive and CT is consistent with a high radiation dose.

Whole body potassium counting is the reference method for measuring body cell mass (BCM) and is only available in a few centres world-wide.

Ultrasound can be used to measure muscle and subcutaneous fat thickness.

1.2.2 Lean body mass

Body protein consists to a large quantity of muscle mass but also of visceral organs and the cells of the blood- and immune system. The body protein is the most metabolically active body compartment and there is a constant protein turnover supplying the body with amino acids to meet changing metabolic demands (185). Structural proteins in connective tissue, fat and bone are not equally exchangeable with other pools of protein (151) but are also components of body protein. The skeletal muscle mass is the body's main exchangeable store of protein and changes in nutritional status, physical activity and disease state have great impact on this compartment. Depletion of LBM with more than 40 % of baseline is not consistent with life (45, 102). Less loss is not fatal but is associated with muscle weakness, disability and decreased energy expenditure as well as with impaired adaptation to metabolic stress and ability to cope with secondary infections and concurrent illness. These effects contribute to increased morbidity and mortality seen in patients with RA (185).

In the literature there are several different definitions of conditions with loss of LBM described (156):

Wasting is the unintentional weight loss affecting both muscle mass and fat mass. It is associated with inadequate energy- and protein intake as in starvation.

The term *cachexia* usually describes a clinical situation with excessive weight loss in the setting of ongoing disease, often with disproportional muscle wasting. With the definition suggested by Roubenoff et al (156), LBM is affected more than fat mass and weight remains stable or increasing due to increase of another body compartment. The abnormalities may for example be extra cellular fluid in renal-, liver- or heart insufficiency or increased fat mass in RA. For these patients dietary intake is usually adequate but the metabolism is altered due to excess production of inflammatory cytokines.

Sarcopenia is the age-associated loss of muscle mass. The etiology is multifactorial with decreasing levels of growth- and sex hormones, physical inactivity, loss of motor neurons and altered motor units activation. Increasing fat mass with increasing levels of

inflammatory cytokines produced by the adipose tissue is also believed to contribute to sarcopenia.

1.2.3 Rheumatoid cachexia

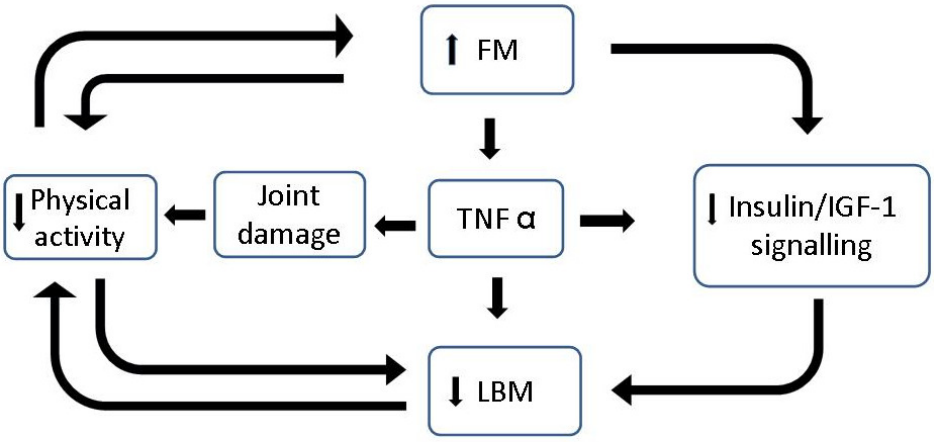
Rheumatoid cachexia is a special form of cachexia seen in inflammatory diseases such as RA and is defined as loss of LBM, predominately skeletal muscle mass, in the presence of stable or increased fat mass with little or no weight loss (185). The prevalence of rheumatoid cachexia depends on the cut-off level for reduced muscle mass and is roughly reported to affect up to two-thirds of patients with RA (158, 159). The average loss of body protein among patients with RA, even in medically well-controlled patients with low to moderate disease activity, is 14%, thus one third of the maximum loss of body protein consistent with survival (158, 184).

Recently, researchers from our group (52) have proposed the definition of rheumatoid cachexia to be fat free mass index (FFMI, kg/m^2) below the 25th percentile of a reference population in combination with fat mass index (FMI, kg/m^2) above the 50th percentile. Rheumatoid cachexia according to this definition is associated with risk factors for CVD, including increased levels of cholesterol and low-density lipoprotein (LDL), a higher frequency of hypertension and metabolic syndrome and decreased levels of the atheroprotective marker anti-phosphorylcholin (anti-PC), findings recently commented by Roubenoff (157).

1.2.4 Mechanisms of rheumatoid cachexia

Pro-inflammatory cytokines and especially TNFα, play a central role in the pathogenesis of muscle protein breakdown in RA (185) and originally, TNFα was called “cachectin”, figure 2.

Figure 2. Mechanisms of rheumatoid cachexia



TNFα causes loss of LBM, predominantly skeletal muscle mass, and reduces physical activity, which reinforces each other as well as predisposes to fat gain. The gain of adipocytes increases production of inflammatory cytokines, which adds to the negative cycle of muscle loss and fat gain. Also, increased FM and TNFα affect the insulin/IGF-1 signalling system.

Insulin and insulin-like growth factor-1 (IGF-1) are anabolic hormones that act through the insulin receptor and further via IRS-1-PI3K/Akt pathway (insulin receptor substrate-1 (IRS-1)-associated phosphatidyl inositol 3 kinase [PI3K]), stimulating protein synthesis and preventing protein degradation. TNFα suppresses this pathway, which results in two important events promoting protein degradation; activation of caspace-3 that cleaves the muscle protein in smaller fragments (actin and myosin) and expression of enzymes that activates the ubiquitin-proteasome system (UPS), which further degrades the proteins to yield amino acids. Besides suppression of insulin- and IGF-1 signalling, TNFα also activates the NFκB (nuclear factor κB) pathway, which is another way to activate protein degradation through the UPS (47).

Reduced physical activity is common in patients with RA and is related to a combination of factors like joint pain and stiffness as well as muscle weakness. Muscle disuse is reported to activate muscle protein cleaving enzymes and the UPS, possibly via calcium overload and increased reactive oxygen species (146). Reduced physical activity also predisposes to adiposity, which further decreases physical activity. Further, increased adipose tissue is associated with increased production of pro-inflammatory cytokines that also contribute to protein degradation as described above (185).

1.2.5 Adipose tissue

Adipose tissue is our primary store of energy. It stores fat as triglycerides (TGs), which consist of 3 free fatty acids (FFA) esterified to one molecule of glycerol. The main source of fatty acids is derived from dietary fat, packed as TGs in chylomicrones (113). TGs are also packed in very low-density lipoproteins (VLDL) secreted by the liver. Lipoprotein lipas (LPL) in capillary walls is activated by apolipoprotein C-II on circulating lipoproteins and hydrolyzes the TGs into FFA and glycerol. The FFAs are re-esterificated to TGs and then stored in the adipocytes.

The adipose tissue responds to available nutrients. After a meal when levels of glucose, chylomicrons and insulin normally are high, re-esterification and storing of TGs in the adipose tissue occur (27). In contrast, in fasting states when levels of available nutrients as well as levels of insulin are low, intra cellular lipolysis by hormone sensitive lipas (HSL) is stimulated, resulting in release of FFA and glycerol (33). Hormones like epinephrine, norepinephrine and glucagon stimulate HSL whereas insulin has an inhibitory effect (33).

Adipose tissue is not just an energy storing organ but has also emerged as an important endocrine organ producing several important molecules such as adipokines, cytokines and growth factors (55). It comprises, besides of the adipocytes (fat cells), of macrophages, endothelial cells, fibroblasts and leukocytes, which make the adipose tissue an important mediator of both metabolism and inflammation (178).

1.2.6 Bone

A hallmark of RA is the bone destruction in the joints. Both local bone erosions and peri-articular osteopenia occur early in the disease (160). Therefore they have achieved important clinical implications as being part of the classification criteria for RA as well as being key variables for monitoring the efficacy of disease modifying anti-rheumatic

drugs (DMARDs). Peri-articular osteopenia and local bone erosions are assessed by X-rays of hands and feet. Besides local bone erosions and peri-articular osteopenia also generalized osteopenia occurs (44), which can be assessed by DXA. The consequences of bone loss are joint destruction with subsequent disability as well as osteoporosis with increased incidence of fractures (44, 88, 171), effects that are associated with increased morbidity and also with increased socioeconomic costs.

To give some information about normal bone loss it is worth mention that bone loss in postmenopausal women is 3% per year the first 5 years after menopause (49). Thereafter the yearly bone loss is about 0.5% (26, 48), which is the same for men above 55 years. The bone loss is usually higher in the metabolically more active trabecular bone compared with cortical bone. A hypothesis explaining this difference is that trabecular bone, which is located mainly in the axial skeleton, contains the bone marrow. Marrow elements are the source of many cells involved in bone turnover and the proximity to these cells results in an earlier and more intense response in the bone remodelling rate (48).

1.2.7 Mechanisms of bone loss in rheumatoid arthritis

Bone tissue has to be responsive to mechanical forces and metabolic regulatory signals and thus undergoes continuous remodelling, a process by which osteoclasts resorb bone tissue and osteoblasts produce new bone matrix that is subsequently mineralised. Bone loss occurs when the balance shifts towards excess resorption (18).

The dominant reason to bone loss in RA is increased bone resorption induced by the osteoclasts (69). The differentiation, activation and survival of the osteoclasts are regulated by the receptor activator of nuclear factor (NF) κB (RANK) – RANK ligand (RANKL) - osteoprotegerin (OPG) signalling (21).

RANK is a membrane receptor expressed on the osteoclasts and triggers a kinase cascade when activated. RANKL is a protein expressed by osteoblasts to coordinate bone remodelling by stimulation of bone resorption by osteoclasts, which in turn stimulate bone synthesis by adjacent osteoblasts. This is a process called coupling and provide balance between bone synthesis and bone resorption (117). However, activated T-cells, synovial fibroblasts and dendritic cells also express RANKL. The known pro-resorptive cytokines IL-1 and TNFα stimulate production of RANKL and increase the potency of RANK-RANKL signalling by up regulating RANK on the cell surface of osteoclasts.

OPG is a protein, also synthesized by the osteoblasts, which has negative impact on RANK-RANKL signalling (81). It is a soluble decoy protein that binds to RANKL preventing its binding to RANK and thereby neutralizes the effect of RANKL (200). Anabolic factors such as estrogens increase the expression of OPG and/or decrease expression of RANKL (86).

The effect of inflammation on bone formation has not been fully investigated but the lack of effective bone formation at erosive sites suggests that the inflammatory process may have a negative impact on the osteoblasts and their function as well (12).

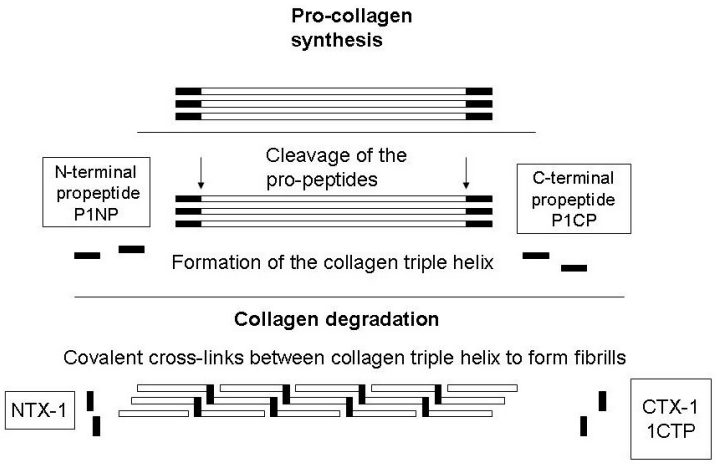
Besides activated pro-inflammatory cytokines, treatment with GCs may contribute to bone loss as well as traditional risk factors for osteoporosis such as age, menopausal status, smoking, low body mass index and physical inactivity (104).

1.2.8 Markers of bone remodelling

Measuring BMD is important in the clinical evaluation of patients with risk for osteoporosis but it represents a static variable, with no insight into the rate of bone turnover. In recent years, biochemical markers of bone turnover have become available and provide a complement to measuring BMD. However, their clinical use has not yet been established although they are suggested to be used in monitoring the effectiveness of anti-resorptive therapy (61). They are also suggested to be used in prediction of bone loss and fracture risk. Individuals with high bone turnover have increased risk of reduced BMD the following years (62) as well as having an increased fracture risk (60). Bone turnover markers are generally inversely correlated with BMD (122).

The primary synthetic product of osteoblasts is type I collagen and several assays against the amino- or carboxy extension peptides of the pro-collagen molecule (P1NP and P1CP) have been developed, figure 3. A disadvantage with these assays is that collagen type I is not unique for bone but is also synthesized by other tissues. Bone-specific alkaline phosphate (BSALP) and osteocalcin are also produced by the osteoblasts and can be used as markers for bone synthesis. BSALP has, however, 10-20% cross-reactivity with the liver isoform and osteocalcin is rapidly degraded in serum (43).

Figure 3. Schematic picture of collagen synthesis and degradation illustrating the origin of some of the different markers of bone turnover.



After the procollagen molecule is secreted extracellular, the amino- and carboxy terminal propeptides are cleaved and released to the circulation where they can be measured as markers of bone synthesis. The collagen molecules form collagen triple helixes that subsequently are joined together by covalent cross-links to form collagen fibrils. When collagen are degraded the different crosslinks are released to the circulation and cleared by the kidney and can be measured as markers of bone resorption.

Most markers of bone resorption are degradation products of collagen type I and can be measured in serum or urine. After cleavage of the pro-collagen extension peptides, formation of the collagen triple helix is followed. The collagen triple helixes are joined together with covalent cross-links to form fibrillar collagen during the maturation process. When mature collagen is degraded these cross-links are released and can be

measured as markers of bone resorption, figure 3. Examples are C-terminal telopeptide crosslaps (CTX-1) and C-terminal telopeptides of type I collagen (1CTP), which are currently considered the best markers for bone resorption (43). CTX-1 and 1CTP respond differently according to the clinical situation (59). CTX-1 has been found in increased levels in patients with postmenopausal osteoporosis and is reduced in response to treatment with bisphosphonates. This is not the case for 1CTP that is not elevated in patients with osteoporosis. Instead 1CTP has been shown to be increased in patients with malignant melanomas, breast- and prostatic cancers. It is also elevated in synovial fluid from patients with RA (164). One explanation for this difference is that CTX-1 and 1CTP fragments are generated through different degradation pathways. CTX-1 is generated by cathepsin K that is selectively expressed by osteoclasts. 1CTP on the other hand is generated by different matrix metalloproteinases (MMPs), whose activity play an important role in collagen degradation associated with RA (124).

1.3 INSULIN-LIKE GROWTH FACTOR-1 (IGF-1)

IGF-1 is an important anabolic peptide and is involved in nutrient transport, energy storing, gene transcription and protein synthesis (130). IGF-1 is important to maintain muscle mass by promoting protein synthesis and inhibiting protein degradation (84). It is also an important bone promoting peptide, stimulating proliferation of preosteoblasts and increases collagen expression while decreasing collagen degradation (120). The action of IGF-1 is mediated mainly by one of two receptors; the type 1 IGF receptor that resembles the insulin receptor with 50% structural homology and the type II IGF receptor (20). IGF-1 can also act on the insulin receptor. Thus, IGF-1 and insulin activate common intra cellular pathways (IRS-1-PI3K/Akt pathway) but with different sensitivities depending on the relative affinities for the receptors and dependent on the abundance of the type of receptor that is present on the target cell (20).

Secretion of IGF-1 from the liver, which is the main source of circulating IGF-1, is regulated by growth hormone (GH) and insulin, which have stimulatory effects (139). Further, nutrition is one of the main determinants of circulating levels of IGF-1 and levels decrease by energy and/or protein deprivation. The sensitivity of circulating IGF-1 to nutrients, the stability of its concentration and its short half-life makes it a useful marker of nutritional status and adequacy of nutritional rehabilitation (177). Besides the liver, IGF-1 is also produced in several other tissues including muscle and bone (37).

Serum levels of IGF-1 decrease with age. Suggested mechanisms for the decrease in IGF-1 with age are lower protein intake, reduced physical activity and decreased levels of growth hormone (GH). These factors are all determinants of IGF-1 and also factors that are often reduced in elderly individuals (142). However, it is not the circulating levels of IGF-1 that are most important for its anabolic action but rather the local concentration of IGF-1 (130).

In the circulation most IGF-1 is bound to binding proteins (IGFBPs). There are 6 different IGFBPs, which can influence the bioavailability, distribution, tissue half-life and cellular action of IGF-1 (11). IGFBP-3 is the most abundant binding protein in the circulation and acts as a reservoir for IGF-1 and also prolongs the half-life of IGF-1 (11). It is produced in several tissues and is regulated by age, nutritional status and GH (20).

IGFBP-1 is mainly produced by the liver and has a strong inverse relationship to plasma insulin and nutritional intake (172). Catabolic and inflammatory conditions with excess production of pro-inflammatory cytokines stimulate production of IGFBP-1 (53). Chronic energy or protein restriction can also increase IGFBP-1 (3, 132, 170). IGFBP-1 is an important regulator of bio-available IGF-1 and has an inhibitory effect on IGF-1 (24, 56). The ratio IGF-1/IGFBP-1 is considered to reflect bio-available IGF-1 (109) and a ratio > 5 indicates an anabolic state (143).

1.4 INSULIN- AND IGF-1 RESISTANCE

Hormone resistance is one of the body's ways to dampen hormone action as well as a way to regulate utilization of potentially limited resources and provide the central nervous system with enough glucose. This adaptive system may help to manage and sustain important processes like; growth and energy storing, reproduction and survival including recovery from injury or infection (130). On the other hand, the negative consequences of insulin resistance are demonstrated in the metabolic syndrome as well as being a known risk factor for development of type 2 diabetes and CVD. As insulin and IGF-1 resemble each other in action, hormone and receptor structure, mechanisms inducing insulin resistance may also induce IGF-1 resistance. Patients with RA have been reported to have reduced peripheral insulin action (138, 155).

1.4.1 Obesity and FFA

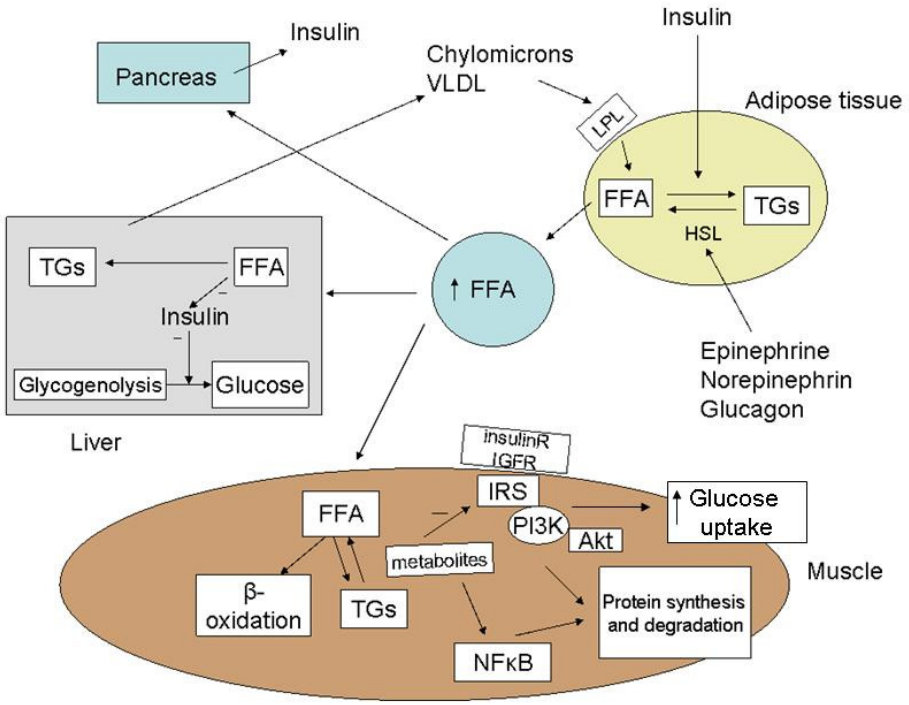
Elevated levels of FFAs play a major role in the development of insulin resistance as reviewed by Boden (15), figure 4. Obese individuals with enlarged FM usually have elevated plasma FFA levels, which have been shown to predict development of type 2 diabetes and to be involved in both peripheral and hepatic insulin resistance.

In the muscle, FFAs are re-esterified to TGs. During the synthesis and breakdown of TGs, different metabolites such as long-chain acyl-coenzyme A and diacylglycerol, are produced. These metabolites activate protein kinase C that inhibits insulin signalling by interfering with the insulin receptor and IRS (91). The metabolites also activate the NFκB pathway, which is a major pro-inflammatory pathway that may play a role in the pathogenesis of CVD (91).

In the liver, FFAs inhibit insulin suppression of endogenous glucose production primary by interfering with insulin suppression of glycogenolysis (16). Both FFA-induced peripheral and hepatic insulin resistance are associated with accumulation of intra muscular and intra hepatic levels of TGs. Increased circulating FFAs stimulate pancreatic β cells to secret insulin (35). Enhanced insulin production compensates for the insulin resistance. However, the long-term effects on insulin secretion have been debated.

Another negative impact of obesity is decreased levels of adiponectin, which also may enhance insulin resistance (94), see below.

Figure 4. Schematic picture of free fatty acids and their role in developing insulin resistance.



FFA = free fatty acids, HSL = hormone sensitive lipas, IGFR = IGF receptor, insulinR = insulin receptor, IRS = insulin receptor substrate-1, LPL = lipoprotein lipas, NFκB = nuclear factor kappa B, PI3K = phosphatidyl inositol 3 kinase, TGs = triglycerides, VLDL = very low-density lipoprotein.

1.4.2 Pro-inflammatory cytokines

One of the major actions of pro-inflammatory cytokines is to restrict growth and energy storing, diverting the resources for survival (130). Thus, TNFα, IL-1 and IL-6 can induce insulin and IGF-1 resistance. The pro-inflammatory cytokines activate several different pathways, which interfere with insulin- and IGF-1 signalling downstream of the receptor and thereby have inhibitory effects on cell proliferation, differentiation and hypertrophy (130).

1.4.3 Glucocorticoids

GC treatment can induce insulin as well as IGF-1 resistance. GCs are steroid hormones essential for survival in times of stress. They are catabolic, increasing the availability of substrates for mitochondrial oxidation and provide the central nervous system with glucose. The effects of GCs are opposite to the effects of insulin and as a result they induce a state of insulin resistance. As IGF-1 and insulin activate common intra cellular pathways (IRS-1-PI3K/Akt pathway) GC excess also has an impact on IGF-1 signalling (20).

1.5 ADIPOKINES

Adipokines (or adipocytokines) are cytokines, mainly produced by adipose tissue. The first adipokine, leptin, was discovered 1994 and thereafter several others have been detected, such as adiponectin, resistin and visfatin.

1.5.1 Leptin

Leptin concentrations are positively correlated with the amount of body fat, which is the main determinant regulating the serum concentration of leptin (55). The most important function of leptin is to inform other organs about the body's energy reserves and especially to signal inadequate energy stores (14). Decreased levels of leptin, or impaired leptin signalling, therefore result in increased appetite and decreased energy expenditure. Unfortunately, body fat excess with increased levels of leptin does not seem to decrease food intake or increase energy expenditure. A suggested mechanism for this is a state of leptin resistance with impaired leptin signalling, especially in hypothalamus (127, 128).

Leptin receptors are expressed in hypothalamus and in several other organs such as pancreas, liver, adipose tissue and the immune system. Besides regulating appetite and energy expenditure, leptin is also involved in regulation of reproduction, growth and immune function (14). For example, leptin is signalling information about adequate energy stores for pregnancy and has been proposed to be a permissive signal for the onset of puberty and maintaining normal fertility (13, 194).

Leptin has several effects on the immune system (178) and acts as a pro-inflammatory cytokine stimulating the macrophages to increased secretion of pro-inflammatory cytokines and up-regulating their phagocytic effects (57). It also affects chemotaxis of polymorphonuclear cells and regulates T helper cells resulting in increased Th1 and suppressed Th2 cytokine production (111). There seems to bee a critical leptin threshold above which increased leptin has no major additional effect on the immune system. Leptin deficiency is on the other hand associated with increased susceptibility to infections (14).

In conditions like sepsis and septic shock leptin synthesis is stimulated by pro-inflammatory cytokines. In these circumstances leptin works more as a stress-related peptide than as a signal about energy stores (55) and it has been proposed that the leptin response is related to the extent of activation of the immune system (8).

1.5.2 Adiponectin

Adiponectin is synthesized mainly by adipocytes but also by skeletal muscle cells, cardiac myocytes and endothelial cells (178). It exists as 3 different isoforms; low molecular weight (LMW), medium molecular weight (MMW) and high molecular weight (HMW) (94). The HMW form is the most abundant form in the circulation and accounts for about 50% of total adiponectin, whereas the other forms constitute about 25% each (63). It has been speculated that the different isoforms of adiponectin have different biological effects.

The serum concentration of adiponectin is negatively associated with BMI and levels of TGs (89, 118) while positively associated to high-density lipoprotein (HDL) cholesterol and apolipoprotein A-1 (118).

Adiponectin interacts with at least two different membrane receptors. Activation of the receptors stimulates the activation of PPARα (peroxisome-proliferator-activated-receptor α) (199) and AMPK (adenosine monophosphate-activated protein kinase) (198), resulting in increased β-oxidation of fat and decreased glucose production in the liver as well as increased β-oxidation of fat and increased glucose uptake in the skeletal muscle. These effects improve insulin sensitivity (94).

Adiponectin has positive effects on endothelial function. It inhibits monocyte adhesion to endothelial cells via inhibiting expression of adhesion molecules and NFκB activation (133, 135). Further, it inhibits the uptake of oxidized low-density lipoprotein (LDL) by macrophages resulting in decreased foam cell formation (134). Adiponectin also has anti-inflammatory properties, inhibiting NFκB activation resulting in modulated signal transduction and decreased levels of pro-inflammatory cytokines. It also enhances the expression of the anti-inflammatory cytokine interleukin-10 (IL-10) (136).

1.6 APOLIPOPROTEINS AND LIPIDS

1.6.1 Apolipoproteins

Low-density lipoprotein cholesterol (LDL) has since a long time been recognized as the primary lipid risk factor for CVD. There are several other atherogenic lipoproteins such as very low-density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and small dense LDL (sdLDL). VLDL transports both triglycerides (TG) and cholesterol and the other lipoproteins transport increasing concentrations of cholesterol.

Apolipoprotein B (ApoB) is present in all the former atherogenic particles with one molecular ApoB in each of the particles. Therefore, apoB reflects the total number of potentially atherogenic particles. ApoB-100 is the dominating apolipoprotein (in the above mentioned lipoproteins) in plasma and is produced in the liver. Besides apoB-100 there is a small amount of apoB-48, synthesized in the gut. ApoB stabilizes and allows transport of TG and cholesterol in plasma and serves as the ligand for the apoB receptors, facilitating uptake of cholesterol in peripheral tissues. It is also apoB that leads to entrapment of these lipoproteins in the arterial wall. More than 90% of all apoB in blood is found in LDL (183).

High-density lipoprotein cholesterol (HDL) has long been recognized as the protective, good cholesterol. Apolipoprotein A-1 (apoA-1) is the major apolipoprotein in HDL particles. ApoA-1 can pick up excess cholesterol from peripheral cells and transport it back to the liver in the HDL particles, so-called reverse cholesterol transport. ApoA-1 also has anti-inflammatory and antioxidant effects. ApoA-1 is not a component in the atherogenic particles but reflects the athero-protective part (183).

There are several advantages determining the ratio ApoB/ApoA instead of the former used ratios such as LDL/HDL or total cholesterol/HDL. LDL is often calculated using the Friedewald formula, based on the determination of total cholesterol, HDL and TG values. Therefore, when calculating the LDL/HDL ratio, HDL is included in both the nominator and the denominator, which seems strange. The same is true when calculating total cholesterol/LDL ratio. In contrast, both apoB and apoA-1 are measured directly and they reflect the two sides of the risk equation and also reflect the balance of cholesterol transport.

1.7 MEDICAL TREATMENT IN RA

1.7.1 General aspects

Today it is well established that an early start with disease-modifying anti-rheumatic drugs (DMARDs) in patients with RA is important to induce remission (126) and to prevent future joint damage (186). Combination therapies are more frequently used and have for many patients been shown to be more effective compared to monotherapy. Methotrexate (MTX) plus sulphasalazine and/or antimalarials and MTX plus TNF inhibitors have particularly favourable benefit/risk ratios (32). Additional treatment with low-dose prednisolone has also been shown to be beneficial (96, 174, 187).

1.7.2 Glucocorticoids (GCs)

Cortisone was first given to a young woman with severe RA. The effect in this patient and 15 other patients was impressive and was reported by Hench in 1949 (83). Systemic corticosteroid treatment was rapidly adopted and used for patients with different rheumatic diseases as well as other disorders like asthma with a similar positive effect. In 1950 the Nobel Prize was awarded for the discovery of the structure and biological effects of the adrenal cortex hormones. However, it was not until 1959 that prednisolone, a cortisone analogue, showed significant beneficial effects compared with aspirin. The outcome measure with the most significant beneficial effect was not a clinical variable but radiological progression of erosions (93). At this time, high doses were given and it was soon discovered that the treatment with GCs was associated with a significant risk of undesirable side effects, which restricted its clinical use. In recent years treatment with GC has been re-evaluated, but in low doses, because of its ability to reduce radiographic damage in early RA (96, 174, 191).

1.7.2.1 Effects on inflammation

GCs are widely used because of their potent anti-inflammatory effects. GC receptors are present in all cell types and GC administration therefore affects most organ systems. GCs diffuse into target cells, in which they are subject to the action of 11β-hydroxysteroid dehydrogenase (11HSD) type 1 or 2, which converts cortisone (inactive form) to cortisol (active form) or vice versa. GC activates the cytoplasmatic GC receptor, which then translocates to the nucleus where it can regulate gene expression. The dominant mechanism for mediating the anti-inflammatory effects is binding of the activated GC receptor to other transcription factors, including NFκB, thereby inhibiting their gene expression. NFκB is activated by pro-inflammatory cytokines and a crucial mediator to development of the inflammatory reaction (121).

1.7.2.2 Effects on lean body mass

GC induced muscle atrophy is characterized by fast-twitch or type II muscle fibre atrophy following protein degradation. GCs activate several components of the UPS (see 1.6.1) and can also activate caspace-3 that degrades myofibrils to smaller fragments. Further, GCs inhibit the effects of IGF-1, which normally inhibits protein degradation. Besides the effects on protein degradation, GCs also have an anti-anabolic effect by decreasing the local production of IGF-1 and stimulating the expression of myostatin resulting in decreased activation of satellite cells, myoblast proliferation and

differentiation (165). There is limited information about GC-induced myopathy in patients with RA but from the scarce information available it is probably rare when low-dose prednisolone (≤ 7.5 mg daily) is used (38).

1.7.2.3 Effects on fat mass

It is well known, already from the first observations of side effects when cortisone was introduced, as well as in patients with Cushing's syndrome that excess of GCs results in a redistribution of body fat from the peripheral fat depots to the abdomen. It is especially observed in patients treated with high doses of GCs but occurs also with low doses (38). The specific increase in intra abdominal fat stores is a consequence of elevated GCs together with increased levels of insulin.

Enzymes within the target cells may limit or amplify the local intra cellular concentrations and effects of hormones. 11HSD type 1 is expressed in several tissues including adipose tissue and especially in visceral adipose tissue. It regenerates cortisol from the inactive metabolite cortisone, thereby amplifying local cortisol concentration. Increased local cortisol concentrations increase expression of LPL and intravascular lipolysis followed by re-esterification and storing of TGs in presence of elevated insulin levels (113). Other suggested mechanisms for the increase of abdominal fat stores are effects of GCs and insulin on stromal fat precursor cells as well as increased abundance of GC receptors on omental adipocytes (39).

GC treatment is also associated with weight gain. In earlier studies with low-dose prednisolone, weight gain of 4-8% has been observed after 2 years treatment (38). One explanation for weight gain is that GCs have major stimulatory effects on food intake (39, 176).

1.7.2.4 Effects on bone

The effects of GCs on bone are dose dependent although there is no dose that is considered entirely safe (38). The most significant effect is inhibition of bone formation caused by depletion of mature osteoblasts through decreased cell proliferation and differentiation as well as an enhanced apoptosis (193). GCs also inhibit the function of differentiated osteoblasts with inhibition of the synthesis of type I collagen, the major component of bone extra cellular matrix with a consequent decrease in bone matrix available for mineralization (42).

The effects of GCs on bone resorption are less clear. GCs seem to have a stimulatory effect in the early phase of osteoclast differentiation, an effect difficult to differentiate from the target of GC treatment i.e. the inflammation. Pro-inflammatory cytokines as well as GCs act by the RANKL pathway and stimulate expression of RANKL and decrease expression of OPG that binds RANKL, preventing RANKL binding to the osteoclast receptor.

Furthermore, GCs decrease calcium absorption in the gastrointestinal system and increase the urinary excretion of calcium (125, 153). This may cause a degree of secondary hyperparathyroidism, but the changes in bone metabolism observed after exposure to GC cannot be explained by this condition. The serum levels of parathyroid hormone are not in the hyperparathyroid range. Further, bone biopsies from patients

with GC induced osteoporosis display decreased bone remodelling in contrast to the increased remodelling seen in patients with hyperparathyroidism (28).

GCs also have a negative impact on the hypothalamic-pituitary-gonad axis. Plasma concentrations of several sex hormones are decreased in both genders taking GCs (154, 161). The actions of GCs and estrogen deficiency on bone loss are additive, which in part can explain the high susceptibility for bone loss in postmenopausal women during GC treatment.

An additional effect of GC is decreased synthesis of local insulin-like growth factors (IGF-I, IGF-II) that are important local regulators of bone cell functions. IGF-I and IGF II are synthesised in the osteoblasts and increase the synthesis of type I collagen and decrease collagen degradation (41).

1.7.3 TNFα

TNF inhibitors have been used for treatment of RA since the late 1990´s. They are included in a new class of anti-rheumatic drugs called the biologics. The name refers to agents with a similarity to endogenous molecules such as antibodies, soluble receptors and antagonistic cytokines. Today there are 3 different TNF inhibitors available as anti-rheumatic drugs; infliximab and adalimumab, which both are monoclonal antibodies against TNF, whereas the third TNF inhibitor, etanercept, is a soluble receptor that binds TNF. As TNFα is central in the pathogenesis of RA it seems important to neutralize the effects of TNFα. There are several studies reporting the efficacy of TNF treatment on disease activity as well as reduced radiographic progression of erosions (98, 110, 192).

1.7.3.1 Effects on body composition

Information about the effects of TNF inhibitors on body composition has not been conclusive. Thus, anti-TNF treatment during 6, 3 and 12 months in patients with early as well as long-standing RA has been reported not to affect body composition. These treatment periods were probably too short to detect significant changes in body composition (115, 123, 168). This suggestion is supported by effects on body composition during longer times in patients with spondyloarthropathy. After 1 year`s treatment with anti-TNF, there was a significant increase in weight and LBM but stable FM (22), while 2 years treatment was associated with a significant increase in weight as well as both LBM and FM (23).

1.7.3.2 Effects on bone

Pro-inflammatory cytokines, such as TNFα and IL-1, are not only involved in the pathogenesis of RA but also in bone loss. They stimulate RANKL expression and activation of the osteoclasts, followed by increased bone resorption (87). The osteoclast is important for all kind of bone loss in RA; local bone erosions, peri-articular and generalized osteoporosis (77). There are several studies showing beneficial effects of TNF antagonists in reducing radiographic progression of erosions (98, 110). Anti-TNF treatment in RA has also been shown to arrest generalized bone loss (78, 108, 196, 197).

2 AIMS

The general aim of this thesis was to study body composition and BMD in patients with RA and how these are affected by inflammation and different treatments of the disease.

Specific aims:

- to study the impact of inflammation and the anabolic factor IGF-1 and its regulating binding protein (IGFBP-1) on body composition, especially LBM, *paper I*
- to study the impact of low-dose prednisolone treatment on body composition and BMD as well as to evaluate the impact in relation to inflammation, physical disability, IGF-1 and biochemical markers of bone turnover, *paper II*
- to study the effects of low-dose prednisolone treatment in patients with early RA on bone remodelling markers in relation to BMD and the association of IGF-1 in relation to bone markers and BMD, *paper III*
- to study if anti-TNF treatment had any effects on body composition and BMD beyond the anti-inflammatory effect in patients with early RA and also the impact on levels of leptin and adiponectin, *paper IV*

3 PATIENTS AND METHODS

3.1 PATIENTS

All patients in the present studies fulfilled the ACR criteria for RA (9).

In paper I, 60 patients (50 women) with established RA, disease duration >2 years, were included when admitted as inpatients to the Department of Rheumatology at Karolinska University Hospital in Huddinge.

In paper II, 100 out-wards RA patients (50% women) were included. Fifty patients had been treated with prednisolone (5-7.5 mg daily) for at least 2 years (the P-group). The other 50 patients were recruited later as a control group and had not been treated with prednisolone during the last 2 years (the NoP- group). They were matched by gender and age (+/- 3 years) with the patients in the P-group. The prednisolone treated patients were recruited between 1996 and 1999. Eighteen of the patients in the control group were recruited during the same time period as the prednisolone treated patients whereas 32 patients were recruited between 2004 and 2006.

This patient population had a high proportion of male patients as a consequence of recruiting the patients from a study on male RA patients.

In paper III, 150 patients (67% women) with early RA (disease duration < 1 year) who had participated in the BARFOT (Better Anti-Rheumatic FarmacOTherapy) low-dose prednisolone study between 1995 and 1999 were included. The patients should have active disease defined as a DAS28 > 3.0. They had been randomized to either the P-group, who were treated with prednisolone 7.5 mg daily (70 patients), or to the NoP-group, who received no prednisolone (80 patients), when they started their first DMARD. The patients were followed for 2 years.

In paper IV, 40 patients with early RA (disease duration <1 year) who participated in the Swefot (SWEdish PHarmacOTherapy) study at Karolinska University Hospital Huddinge were included between 2003 and 2005. These patients all started treatment with methotrexate (MTX) up to 20 mg/week. Those who, after 3 months, still had active disease defined as DAS28 >3.2, were randomized; treatment A: MTX with addition of SSZ, 2000 mg/day, and HCQ, 400 mg daily, and treatment B: MTX with the addition of the TNF antagonist infliximab (INF), 3mg/kg body weight given intravenously at weeks 0, 2, 6 and every 8 weeks thereafter. The patients were followed for 2 years.

3.1.1 The BARFOT and Swefot studies

In this thesis I have used data from two Swedish multi-centre studies.

BARFOT (Better Anti-Rheumatic FarmacOTherapy) is a Swedish multi-centre observational study designed to investigate clinical and therapeutic aspects of early RA (disease duration < 1 year). A sub study was designed to compare the addition of prednisolone 7.5 mg daily to DMARD therapy with DMARD therapy alone (174). The primary end point was the difference in changes in radiographic damage scores after 2 years and the secondary end points were remission rates and differences in disease activity and function. The randomization was done as block randomization for each

centre according to a central randomization program with stratification for gender. Inclusion criteria were RA according to the ACR criteria (9), age between 18 and 80 years, disease duration less than 1 year and active disease defined as a DAS28 >3.0. Exclusion criteria were earlier treatment with glucocorticoids (GC) or any contraindications for GC therapy. Patients with previous fragility fractures as well as patients <65 years with a T-score < -2.5 or patients >65 years with a Z-score < -1.0 on DXA, were also excluded.

Swefot (Swedish pharmacotherapy) is an open Swedish multi-centre randomized study designed to compare two treatment strategies in patients in whom MTX up to 20 mg/week had not lowered Disease Activity Score of 28 joints (DAS28) to ≤ 3.2 during the first 3 months of disease treatment (190). After 3 months the patients who had not achieved low disease activity were randomized; treatment A: MTX with addition of SSZ, 2000 mg/day, and HCQ, 400 mg daily, and treatment B: MTX with the addition of the TNF antagonist infliximab (INF), 3mg/kg body weight given intravenously at weeks 0, 2, 6 and every eight weeks thereafter. Patients were randomized according to a computer generated random list, kept at the study centre. Inclusion criteria were RA according to the ACR criteria (9), age 18-80 years, disease duration less than 12 months and active disease defined as DAS28 of more than 3.2. Exclusion criteria were prior DMARD therapy or contraindication to any of the trial medications, malignancy, current infection, pregnancy or decreased liver or renal function.

3.2 METHODS

3.2.1 Disease activity

Disease activity was assessed by the composite index Disease Activity Score of 28 joints (DAS28). It contains the tender joint counts (TJC) of 28 joints, the swollen joint counts (SJC) of 28 joints, the erythrocyte sedimentation rate (ESR) and the patients global assessment of general health (GH) measured on a visual analogue scale (1-100 mm)(148). It is calculated with the following formula: DAS28= 0.56*sqrt (TJC) +0.28*scrt (SJC) + 0.70* ln (ESR) +0.014*GH.

A DAS28 level > 5.1 corresponds to high disease activity, > 3.2 and ≤ 5.1 with moderate disease activity and < 3.2 represents low disease activity. A patient is regarded as being in remission if DAS28 is below 2.6 (189).
In paper III active disease was defined as DAS28>3.0.

Disease activity was also assessed by C-reactive protein (CRP), paper I, III and by high sensitive CRP, paper II.

3.2.2 Functional capacity

Functional capacity was measured using the Swedish version of the Stanford Health Assessment Questionnaire (HAQ), which is a self-reporting formula (50). It consists of 20 questions, divided into 8 categories, each consisting of 2 to 3 activities of daily living: dressing and grooming, arising, eating, walking, hygiene, reach, grip and other common daily activities. The response to each question ranges from 0 (without any difficulty) to 3 (unable to do). The created disability index ranges from 0 to 3.0, where a higher score indicates a higher degree of disability (54). Increased HAQ-score is a consequence of both inflammation and joint destruction (73, 167). During the first 5

years of disease, inflammation is considered to be the main contributor to disability, whereas joint damage has more impact later on.

A disadvantage with the HAQ disability index is the lack of sensitivity at the ends of the spectrum. A person with a HAQ-score of 0 (not disabled) may have difficulty performing more advanced activities and may get better whereas a person scoring 3 (completely disabled) could still get worse. Further, we do not know if the disability distance between 0 and 1 is the same as between 1 and 2 or between 2 and 3 (25).

3.2.3 Body composition and BMD

BMI was calculated by dividing body weight by the square of height (kg/m^2). Values between 18.5-24.9 kg/m^2 are considered normal, values <18.5 kg/m^2 correspond to underweight, values between 25-29.9 to overweight and values >30 kg/m^2 to obesity.

Body composition was measured by total body dual-energy x-ray absorptiometry (DXA), GE-Lunar Prodigy, software enCore 2006, version 10, 20,105. Lean body mass (LBM), fat mass (FM), bone mineral content (BMC) and bone mineral density (BMD) were assessed. Fat free mass (FFM) was the sum of LBM and BMC and expressed in absolute kilograms. FM was expressed in kilogram, percentage of body weight and as a Z-score (the number of SDs from the mean in healthy age-and sex-matched people, values obtained from Lunars combined European/US reference population). The reference value for FM% is for women 20-30% and for men 12-20% (1). Overweight is defined as FM >33% in women, >25% in men. Obesity for individuals younger than 60 years is defined as FM >41% for women, >29% for men and for individuals older than 60 years >43% for women and >31% for men (58).

Fat free mass index (FFMI, kg/m^2) and fat mass index (FMI, kg/m^2) were calculated. Data from a Swiss population of healthy adults (2986 men, 2649 women) were used to classify the patients as under lean or having excess fat mass (106, 166). Cut-off values for being under lean were defined as FFMI below the 10th percentile and obesity was defined as FMI above the 90th percentile.

The patients were categorized as rheumatoid cachectic if FFMI was below the 10th percentile and FMI above the 25th percentile and as wasted if FFMI was below the 10th percentile and FMI below the 25th percentile.

BMD values were given in grams of bone mineral per square centimeter (g/cm^2) and as Z-scores (the number of standard deviations (SD) from the mean of healthy age- and sex-matched people) and T-scores (the number of SDs from the mean in healthy young sex-matched people). Osteoporosis was defined as T-score ≤ -2.5 SD.

3.2.4 Assays of serum samples

In paper I serum samples were collected after an overnight fast. In paper II-IV serum samples were obtained between 9 am and 3 pm. All serum samples, which were not analysed directly, were stored at -70°C until assay.

In paper I the assays included: C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), glucose, insulin, IL-6, TNFα, IGF-1, IGFBP-1, albumin, cholesterol, HDL, LDL, triglycerides and RF. The present values of glucose were suggested to be slightly lower than expected, as glucose was analysed in frozen serum.

In paper II the assays included high sensitive CRP, ESR, IGF-1 and markers of bone turnover (P1NP, CTX-1, 1CTP).

In paper III samples included CRP, ESR, IL-6, IGF-1 and markers of bone turnover (P1NP, CTX-1, 1CTP).

In paper IV samples included ESR, markers of bone turnover (P1NP, CTX-1, 1CTP), IGF-1, apoA-1, apoB, adiponectin and leptin.

ESR, CRP, albumin, cholesterol, HDL, LDL and triglycerides were analysed directly using standard laboratory methods with commercial kits.

3.2.4.1 Cytokines

TNFα and IL-6 were analysed at the Study centre for laboratory medicine, Karolinska University laboratory, Stockholm, Sweden, and determined by the commercial kits Quantkine® HS TNFα and IL-6, respectively, which are solid-phase enzyme-linked immunosorbent assays (ELISAs) that employ the quantitative sandwich enzyme immunoassay technique. The mean and range of TNFα in healthy persons are 2.07 pg/ml and <0.12-4.71 pg/ml, respectively. For IL-6 the corresponding figures are 1.77 pg/ml and 0.447-9.96 pg/ml.

3.2.4.2 Anabolic factors

The anabolic factors were analysed at Centre for Diabetes Research, Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden.

IGF-I and IGFBP-1 were determined by radioimmunoassay (RIA) (10, 147). The mean (SD) of IGFBP-1 in a Swedish normal population of 274 individuals is 24.3 (18.6) µg/L for men and 32.5 (22) µg/L for women (181). The ratio IGF-1/IGFBP-1 was calculated as it reflects the concentration of bioavailable IGF-1(109). A ratio of IGF-1/IGFBP-1 exceeding 5 indicates an anabolic state (143). As serum levels of IGF-1 are age dependent, decreasing which age, IGF-I values were also expressed as SD scores calculated from the regression of the values of 247 healthy adult subjects (85).

Insulin was determined by ELISA using a commercial kit (DakoCytomation Insulin K6219). The reference interval is 1.8-14.3mU/L (=11-86 pmol/L) (95% confidence interval). Insulin resistance was assessed by calculating the homeostasis model assessment of insulin resistance (HOMA-IR) by the formula: fasting insulin* fasting glucose/22.5 (119). Hepatic insulin resistance was calculated from fasting insulin * IGFBP-1, reference value is mean(SD) 106(27) (150).

As IGFBP-1 and insulin must be analysed from fasting blood samples, we only analysed these in paper I.

3.2.4.3 Adipokines

Leptin and adiponectin was analysed at the Centre for Diabetes Research, Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden and determined by (RIA) by HL-81K from Linco and HADP-61 from Linco and Millipore, respectively. Normal mean (SD) leptin values (BMI ranges 18-25) are for lean men 3.8 (1.8) µg/L and for lean women 7.4 (3.7) µg/L. Levels rise approx 2.5 times faster in women per unit BMI as compared to men (112). Normal mean (SD) levels of

adiponectin from 205 Swedish healthy adults (106 women, 99 men) are for women 11.88 (4.69) mg/L and for men 7.34 (3.55) mg/L, personal communication.

3.2.4.4 Apolipoproteins

The apolipoproteins were analysed at the Study centre for laboratory medicine, Karolinska University Hospital, Stockholm, Sweden. Apolipoprotein A1 (apoA1) and apolipoprotein B (apoB) were determined by Synchrone LX from Beckman AB by turbidimetry. The reference interval for apoA-1 is for women 1.10-2.10 g/L and for men 1.10-1.80 g/L. The reference interval for apoB is for individuals' younger than 40 years 0.50-1.50 g/L and for individuals 40 years and older 0.50-1.70 g/L. The ratio ApoB/ApoA1 was calculated. A ratio > 0.6 for women and > 0.7 for men is considered a moderate risk for CVD and the risk is increasing almost linearly with increasing ratio. For patients with other risk factors of CVD the preferable ratio should be even lower (183).

3.2.4.5 Markers of bone turnover

The bone markers were analysed at the Study centre for laboratory medicine, Karolinska University laboratory, Stockholm, Sweden. Procollagen type I N-terminal propeptide (P1NP) was used as a marker of bone formation, and C-terminal telopeptide crosslaps (CTX-1) and C-terminal telopeptides of type I collagen (1CTP) as markers of bone degradation.

P1NP was determined by Roche Elecsys 1010/2010 total P1NP serum kit, which employs the electrochemiluminescence immunoassay (ECLIA) technique. The reference intervals are for premenopausal women <60 μg /L, for postmenopausal women <80 μg /L and for men <45 μg/L.

CTX-1 was determined by Roche Elecsys 1010/2010 β-CrossLaps serum kit, which also employs the ECLIA technique. The reference interval is for premenopausal women <570 pg/mL and for postmenopausal women <1000 pg/mL. For men the reference interval is <580 pg/mL if < 50 years of age, <700 pg/mL if between 50-70 years old and < 550 pg/mL if > 70 years old.

1CTP was determined by Orion Diagnostica RIA technique. The reference interval is 1.8-5.0 μg/L.

3.3 STATISTICAL ANALYSES

All the statistical analyses were performed with STATISTICA 7 or 8 from Stat Soft Scandinavia AB, Tulsa, Oklahoma, USA. Data were presented as mean (95% confidence interval, CI) for normally distributed variables or median (interquartile range, IQR) for nonparametric (not normally distributed) variables, *paper I, II and IV*. In *paper III* normally distributed variables were presented as mean (standard deviation, SD). Comparisons between groups were made with the Mann-Whitney U-test for nonparametric variables or with Students t-test for normally distributed variables. For differences between groups for binary or categorical data, Fishers exact test or χ^2 - test were performed. χ^2 – test was used when all of the expected values were at least 5; otherwise Fishers exact test was used. Correlation analysis was performed with Spearman's rank order correlations. Multivariate analyses were performed with

multiple linear regression analyses. In *paper I* the multivariate regression model included at most 4 variables at the same time due to the relatively small sample size; in *paper IV* the model included at most 2 variables at the same time. In paper I the dependent variable LBM was log-transformed to obtain normal distribution. Comparisons between two repeated measures within the same group were performed by T-test for dependent samples or Wilcoxon matched pairs test, depending on the distribution, *paper III and IV*. To adjust for differences between the groups we used ANCOVA (analysis of covariance) in *paper III.* In *paper IV* we calculated the area under the curves (AUCs) according to the Trapezoidal role for several variables from time point 0, 3, 12 and 24 months. Differences between means from reference materials and our results were computed by difference tests, *paper I and IV*. In paper IV, only patients that continued the respective protocol treatment were included in the analyses. Intention-to-treat was thus not applicable in this mechanistic study. In all papers p-values <0.05 were considered significant, meaning that there is less than 5% chance that the result is just a coincidence. P-values <0.1 are sometimes reported as trend values.

4 RESULTS AND DISCUSSION

4.1 BODY COMPOSITION (I, II, IV)

4.1.1 Lean body mass

A large proportion (50%) of the in-ward RA patients with long disease duration and active disease had low fat free mass (defined as FFMI < the 10^{th} percentile of a reference population), *paper I*. The proportion in outpatients with established RA was 38%, *paper II* and in the group of patients with early RA it was 19%, *paper IV*. In this longitudinal study of patients with early RA, all patients included, LBM increased over the 2 years, +1.0 kg (95%CI 0.3-1.8), p=0.009, *paper IV*.

BMI was low (<18.5 kg/m^2) in only 10% of the in-ward RA. In the established out-ward RA patients, none had low BMI and in the patients with early RA only one had low BMI. Thus, BMI is not a reliable method to detect low muscle mass.

In the longitudinal studies of early RA (III and IV), all patients included, BMI increased significantly after 2 years, +0.72 (95% CI 0.51-0.94) kg/m^2, p<0.001 in paper III and +0.64 (95%CI 0.11-1.18) kg/m^2, p=0.019 in paper IV.

In multiple regression analyses (paper I) we found that, besides gender, low bioavailable IGF-1 (beta= 0.41, p<0.001), high HAQ-score (beta= -0.31, p=0.006) and high DAS28 (beta= -0.21, p=0.030) were independently correlated with low muscle mass assessed as LBM. This model explained 42% of the variance of LBM. In paper II, only CRP correlated with LBM, (beta -0.13, p= 0.033), besides gender (beta 0.87, p<0.001). In paper IV there were no significant correlations with LBM.

It was not surprising that the highest prevalence of low muscle mass was found in the group of RA patients who had the highest disease duration and disease activity. Important contributing factors to the observed low muscle mass was inflammation, measured as DAS28 but also as HAQ-score, which reflects long-term inflammatory burden. HAQ-score was also our surrogate variable for physical activity as higher HAQ-score after the first 5 years of disease mainly is a consequence of joint destruction, regularly followed by decreased physical activity (131). Our findings are in concordance with previous reports (185).

A new finding was the association of high IGFBP-1 with low muscle mass. IGFBP-1 is an important regulator of bioavailable IGF-1 and it is stimulated by pro-inflammatory cytokines. We found that the ratio IGF-1/IGFBP-1, reflecting bioavailable IGF-1, was low indicating a catabolic state, which contributed to the reduced LBM in these patients.

About one third of the patients with low to moderate disease activity from the out-patient clinic also had low FFM. This is concordant with medically well controlled RA patients, who had 14% less BCM compared with healthy controls (158, 184). A possible explanation is that the decrease in LBM is a result of several years of disease with varying inflammatory and physical activity. This explanation is confirmed by the finding that in patients with early RA and intensive treatment, paper IV, only less than 20% had low muscle mass at the 3 months visit and after 2 years there was indeed an increase in LBM. Thus, inflammatory control in combination with less disability secondary to early, aggressive treatment seems to be important. To stop wasting muscle

mass, the inflammatory activity must be controlled but that may not be enough. To get an increase in skeletal muscle mass, high-intensive resistance training must be performed (116) and the intake of protein must be adequate (114).

4.1.2 Adipose tissue

BMI was above normal (>25 kg/m^2) in 45-50% of the patients. Many of the RA patients had high fat mass. FMI was above the 90th percentile of the reference population in 34-45% of the patients with the lowest percentage in patients with early RA and the highest percentage in the in-ward RA patients. In all groups of RA patients, the proportion of patients with FM% corresponding to overweight or obesity was 80%. In the longitudinal study of early RA, all patients included, there was an increase in FM, +1.6 (95% CI 0.4-2.9) kg, p=0.012, after 2 years, paper IV.

The trunk: peripheral fat ratio was high, indicating central obesity in both study II, 1.04 (0.33) in women and 1.37 (0.33) in men and study IV, 1.09 (0.22) in women and 1.67 (0.33) in men.

In multiple regression analyses in paper II, we found that a higher HAQ-score (beta 0.20, p=0.043) and GC treatment (beta -0.21, p=0.035) correlated with higher FM, whereas smoking (beta -0.24, p=0.015) correlated with less FM. In multivariate analysis in paper IV, we found that treatment with TNFα inhibitors was significantly correlated with FM gain from 3 to 24 months (beta=0.43, p=0.034).

In univariate analyses in paper I and IV, there were no significant correlations between markers of inflammation or HAQ-score and FM. However, in study II, we found a positive correlation between CRP and FM, but only in women. There were expected positive correlations between FM and insulin (p<0.001) and HOMA (p=0.002), paper I.

Increased FM and abdominal obesity in RA patients have been described earlier, both in longstanding RA (51, 65, 90, 195) as well as in early RA (19). One possible explanation has been that impared physical activity contributes to increase of adipose tissue. This suggestion is in line with the found positive correlation between HAQ-score and FM in paper II, as HAQ-score may be a surrogate variable for physical activity. Also Giles et al (65) found an association between FM and HAQ-score as well as variables of disease activity such as CRP. However, other groups have not found such correlations (19, 195). The reason for increased FM in RA is thus hitherto partly unsolved.

Adipose tissue is a potent source of inflammatory cytokines that can induce CRP production and contribute to systemic inflammation. Thus, an association between FM and serum levels of CRP has been shown in the general population (71). Such positive correlation between FM and CRP was found also in women but not men with RA, paper II, which is similar to the findings by Giles et al (64). However, such correlation was not found in study I and IV. It must thus be remembered that CRP production may be a consequence of increased FM and not only a marker of the rheumatoid inflammatory process.

4.2 BONE

4.2.1 BMD (I-IV)

In the studies with longstanding RA (I, II), the proportions of patients with osteoporosis according to T-scores were 28% and 26%. In paper IV, the corresponding figure was 9% at the 3 months visit. In paper III, low bone mass (defined as a T-score below -2.5 SD for patients <65 years or a Z-score below -1.0 for patients >65 years), was a contraindication to be included in the study. However 50% of the patients had osteopenia according to T-scores.

In paper III, BMD decreased significantly during the 2 years study period. The decrease was significant for both treatment groups at lumbar spine, -2.8%, p<0.001 in the P-group and -1.1%, p=0.034 in the NoP-group, and for the NoP-group also at femoral neck, -1.9%, p<0.001. In contrast, in paper IV, there was no loss of bone in any of the treatment groups during the study period. In fact there was an increase in BMD at lumbar spine after 2 years in the anti-TNF treated group, +3.1 (95% CI 0.2-5.9) %, and an increase at femoral neck after 1 year in the DMARD combination group, +1.1 (95% CI -0.0-2.3) %.

In paper 1, 9 women and 1 man (total 17%) were treated with bisphosphonates. In paper II, 29% of the women, all post-menopausal, had treatment with bone resorption inhibitors, either hormone replacement therapy or bisphosphonates. None of the men had anti-resorptive treatment. None of the patients in paper III were treated with bone resorption inhibitors. Two of the patients in paper IV were treated with bisphosphonates.

In multiple regression analysis in paper II, we found that weight was positively correlated with BMD at both lumbar spine and femoral neck (beta 0.33, p<0.001 and beta 0.42, p<0.001, respectively). Age correlated negatively with BMD at both skeletal sites (beta -0.21, p=0.030 and beta -0.39, p<0.001, respectively). Smoking was negatively correlated with BMD at lumbar spine (beta= -0.20, p=0.032), whereas HAQ-score was negatively correlated with BMD at femoral neck (beta= -0.22, p=0.012).

In multiple regression analyses in paper III, markers of bone turnover (P1NP) and CRP at 12 months were negatively correlated with BMD at lumbar spine (beta= -0.26, p=0.009 and beta =-0.25, p=0.006, respectively), whereas IGFSD at baseline correlated positively with BMD (beta 0.17, p=0.059). GC treatment had a negative impact on BMD at lumbar spine (beta=0.21, p=0.036).

At femoral neck, BMI was positively correlated (beta= 0.31, p<0.001), whereas markers of bone turnover (1CTP) and CRP at 12 months were negatively correlated with BMD (beta = -0.26, p=0.006 and beta = -0.20, p=0.017). GC treatment had no impact on BMD at femoral neck.

More than one fourth of the patients with longstanding disease had osteoporosis. This is more compared with a general healthy population. In a study by Haugeberg et al (80), the overall proportion of osteoporosis among female RA patients was 14.7-16.8%, which is a 2-fold increase of osteoporosis compared with a reference population of healthy individuals. We did not perform any sub group analysis of the frequency of osteoporosis but it generally increases with age and also with menopausal status. There

are several reasons to the higher frequency of osteoporosis in RA patients; traditional risk factors such as older age and low weight but also risk factors associated with the RA disease, such as inflammation, disability and treatment with GCs (80). Our results from the multiregression analyses are in concordance with those earlier described risk factors and further strengthen the importance of decreasing disease activity and preventing disability.

Our results from paper III showed that BMD loss at femoral neck in the NoP-group (-1.9%) was higher compared with a general Dutch population >55 years where bone loss at femoral neck was 0.4% in men and 0.6% in women after 2 years (26). In previous longitudinal studies in RA, BMD changes ranged from -0.3% to -2.4% in lumbar spine and from -0.1% to -4.3% in the hip (4, 34, 70, 79, 169). The result from paper IV, with no loss of bone, indicates that early intensive treatment prevents bone loss.

4.2.2 Markers of bone turn-over (II-IV)

In all papers, the median levels of the markers of bone turnover were within the reference ranges, except for 1CTP, which was slightly elevated in the P-group in paper II. The marker of bone turnover that was elevated in the highest proportion of the patients was 1CTP. It was elevated in 38% of the patients in paper III, 39% in paper II and 43% in paper IV.

In both paper II and III, markers of inflammation were significantly and positively correlated with markers of bone turnover. The highest regression coefficient was found between 1CTP and CRP ($r = 0.57$, $p<0.001$ in paper II and $r=0.48$, $p<0.001$ in paper III).

Baseline levels of IGF-1 correlated negatively with both CTX-1 and 1CTP ($r= -0.21$, $p=0.012$ and $r =-0.38$, $p<0.001$, respectively), paper III. Baseline levels of IGF-1 did not correlate with baseline levels of any of the markers of bone turnover in paper IV. In paper II there was a positive correlation between IGF-1 and BMD at femoral neck ($r= 0.22$, $p=0.039$).

In paper II, there was a negative correlation between CTX-1 and BMD at lumbar spine, $r = -0.25$, $p=0.016$, whereas in paper IV, we found a negative correlation between CTX-1 and BMD at femoral neck, $r = -0.32$, $p=0.043$ and a trend for a negative correlation between P1NP and BMD at lumbar spine, $r = -0.25$, $p=0.059$. We could not find any correlations at baseline between markers of bone turnover and BMD at lumbar spine or femoral neck in paper III.

The finding that particularly 1CTP was increased in a large proportion of the RA patients, is in concordance with earlier reports of elevated levels of 1CTP in serum and synovial fluid in RA patients (164). Also, the finding of a positive correlation between 1CTP and markers of inflammation is in line with earlier reports (164). Further, the negative correlation between markers of bone turnover and BMD is in concordance with earlier reports (122).

Changes in markers of bone turnover in the two prospective studies are discussed in section 4.3.3 and 4.4.3.

4.3 EFFECTS OF GLUCOCORTICOID TREATMENT (II, III)

4.3.1 Effects on LBM (II)

In the cross-sectional study assessing treatment with low-dose prednisolone in established RA we did not find any significant difference between the treatment groups in variables of muscle mass. However, when separated for gender, we found that men in the prednisolone treated group had significantly lower FFMI compared with men not treated with prednisolone, 17.7(1.4) kg versus 18.7 (2.1) kg, p=0.016. We believe that this difference may be related to a more severe disease in the prednisolone treated group and not to treatment with GCs. This hypothesis is supported by the results from the multi regression analyses where we found a correlation between CRP and LBM as well as with FFM, whereas treatment with GCs had no impact on these variables.

A well known effect of GC treatment is a negative impact on the hypothalamic-pituitary-gonad axis with reduced levels of testosterone, which is a known muscle promoting hormone in men. An effect on body composition with decreased levels of LBM has been shown in men treated with androgen deprivation due to prostatic cancer (188). However, in study II such impact on testosterone levels was probably not present, as there was no impact of GC treatment on LBM. There are limited data in the literature of steroid induced myopathy but it is probably very rare with GC doses ≤7.5 mg daily (38).

4.3.2 Effects on FM (II)

In paper II, patients treated with low-dose prednisolone had significantly higher fat mass compared with those not treated with prednisolone, (FM = 27.6 (8.6) kg versus 23.1 (8.6) kg, p= 0.012). All patients, irrespective of GC treatment, had a high trunk: peripheral fat ratio indicating abdominal obesity. However, there was no significant difference in this ratio between the treatment groups. In multiple regression analysis we found that treatment with GCs correlated significantly with high fat mass (beta -0.21, p=0.035).

We had expected that the trunk: peripheral fat ratio should be higher in GC treated patients as redistribution of body fat to the abdominal region is a known side effect of GC treatment. The same negative finding has also earlier been described concerning low-dose prednisolone treatment (90). Thus, the prednisolone dose was probably too low to further increase abdominal fat in patients already centrally obese.

4.3.3 Effects on bone (II, III)

In paper II, there were no significant differences between the treatment groups in BMD, neither at the lumbar spine (p= 0.91) or femoral neck (p=0.50). In multiple regression analysis, treatment with GC had no impact on BMD at lumbar spine or femoral neck. This finding is in contrast to earlier cross-sectional studies (75, 80) in which BMD was reduced and independently associated with the use of GCs in RA women compared with healthy controls and a reference population. Neither in these studies, nor in the present were the patients randomized to GC treatment, which implies possible confounding by indication. One explanation why BMD was not decreased in the P-group compared with the NoP-group, may be the high frequency of bone resorption

inhibitor therapy (30%) in the RA women that may have prevented bone loss. Another explanation might be the overweight in the present patients, which was also confirmed in the correlation analyses.

In study II, where the patients in the P-group had been treated with GCs since at least 2 years, there were no significant differences in P1NP or CTX-1 between the treatment groups. However, 1CTP was significantly higher in the P-group, 5.1 (4.2-7.4) µg/L versus 4.4 (3.7-5.6) µg/L, in the NoP-group, p=0.041.

The most significant effect of GC on bone is inhibition of bone formation and subsequent inhibition of the synthesis of type I collagen. Decreasing levels of bone formation markers have been reported when starting GC treatment (101). In study II we did not see any significant difference in serum levels of P1NP between the treatment groups. This confirms the lack of significant differences in markers of bone formation in RA patients treated with GC for a longer time compared with patients not treated with GC (103). It thus seems likely that the decrease in bone formation is observed mainly during the first months after starting GC treatment. The higher 1CTP found in the P-group was probably depending on a higher disease activity in this group as shown by the higher levels of CRP as well as HAQ-score. This suggestion is supported by the significant correlations found between markers of bone resorption and inflammatory activity.

In paper III, BMD at the lumbar spine decreased significantly after 2 years in both treatment groups (-2.8%, p<0.001 in the P-group and -1.1%, p=0.034 in the NoP-group). The reduction in BMD was not significantly different between the treatment groups. However, reduction in Z-score at the lumbar spine was significantly different, (-0.30 in the P-group and -0.10 in the NoP-group, p=0.043). On the other hand, BMD at the femoral neck remained stable in the P-group (-0.75%, p=0.20), while it decreased significantly in the NoP-group (-1.9%, p<0.001). This reduction was though not significantly different between the treatment groups.

As age and proportion postmenopausal women unfortunately differed between the treatment groups, we also performed the statistical analyses with women around menopause excluded (age between 47 and 52 years, totally 16 women). The reductions in BMD and Z-scores at the lumbar spine were then not significantly different between the treatment groups. However, when looking at only women, in spite of those around menopause excluded, there were significant differences between the treatment groups in BMD and Z-scores at the lumbar spine, (-3.8% in the P-group versus -0.3% in the NoP-group, p=0.004 for changes in BMD and -0.30 in the P-group versus 0.00 in the NoP-group, p=0.007 for changes in Z-scores). No difference between the treatment groups was shown for premenopausal women or for men.

If the study in paper III had been conducted today probably a T-score below -1.0 SD had been a contraindication as treatment with bisphosphonates is recommended for GC treated patients with a T-score below -1.0 SD. Further, supplement with both calcium and vitamin D are recommended when treatment with GCs is prescribed, in contrast to only supplement with calcium at the time the study was conducted. Almost 50% of the GC treated patients had osteopenia and should today have got prophylactic treatment with calcium and vitamin D supplement as well as bisphosphonates, which probably had prevented some of the bone loss in both groups.

In paper III, there were significant differences concerning the changes for all the bone markers. The decrease in bone formation occurred only in the P-group. The decrease in bone resorption markers were significantly more pronounced and occurred earlier in the P-group.1CTP and CTX-1 decreased with different patterns in the two treatment groups, which may reflect different resorption processes. 1CTP is preferentially localized in local joints and is generated by MMPs (164). 1CTP has been associated with a more aggressive joint disease (74) and suggested to be a predictor of a more erosive disease course (5, 137). The greater reduction of 1CTP found here in the P-group indicates a more pronounced inhibition of MMPs induced by GCs. This inhibition might not only explain the earlier reported ability of prednisolone to reduce radiographic damage in the joints of hands and feet in the RA patients (174) but also the finding that bone resorption was only inhibited in the P-group in the femoral neck, a juxta-articular localization.

4.4 EFFECTS OF ANTI-TNF TREATMENT (IV)

4.4.1 Effects on LBM

TNF treatment had no impact on LBM. The patients treated with anti-TNF had well preserved LBM during the 24 months study period.

The reason for lack of increased muscle mass when disease activity decreased is probably dependent on that only 20% of the patients had low muscle mass from start in this cohort of early RA. On the other hand, patients treated with the combinations of DMARDs had a significant increase in LBM after 12 months, 0.8 (-0.1-2.2) kg, p= 0.030 versus 0.4 (-0.6-1.3) kg, p= 0.43. However, changes in LBM or FFM did not differ significant between the treatment groups.

Our result, with no change in LBM during anti-TNF therapy, is in concordance with two earlier reports, where the treatment durations were shorter (123, 168). However, the study of Briot et al (23) in patients with established spondylarthropathy showed an increase in LBM after 2 years treatment with etanercept, which might depend on that LBM was low from start. Further, in a subgroup of patients with established RA who gained weight during a follow-up of a randomized study, significantly more was FFM in the anti-TNF treated group compared with patients treated with MTX (115). Also these patients had probably low FFM from start. It thus seems likely that intensive reduction of inflammation during a long period might increase reduced muscle mass in patients with arthritis.

4.4.2 Effects on FM

Anti-TNF treated patients had a significant increase in FM at the 12 months visit compared with the patients treated with the combination of DMARDs, +1.2 (0.1-2.3) kg, p=0.037 versus +0.5 (-0.6-1.7) kg, p=0.32. The increase in FM was sustained and further aggravated at the 24 months visit, +3.8 (1.2-6.4) kg, p=0.009 versus +0.4 (-1.7-2.5) kg, p=0.72. The increase in FM after 24 months was valid for all variables of fat mass. The changes in FM from 3 and 24 months were significant different between the treatment groups, p=0.034.

In multi regression analysis we found that treatment with anti-TNF correlated significantly with FM gain between 3 and 24 months (beta=0.43, p=0.034). Thus, the

increase of FM seems to be a specific effect of anti-TNF treatment. This has not been shown previously in RA with shorter treatment periods (115, 123, 168), but similar results have been reported in patients with spondylarthropathy during 2 years treatment with TNF antagonists (23).

4.4.3 Effects on bone

There was an increase in BMD at lumbar spine after 2 years in the anti-TNF treated group, + 3.1 (95% CI 0.2-5.9) % and an increase at femoral neck after 1 year in the DMARD combination group. However, the increase in femoral neck was not sustained at the 2 years visit. There were no significant differences between the treatment groups in changes in BMD at either skeletal site between the randomization and the 12 and 24 months follow-ups. There was no bone loss in any of the treatment groups during the 2 years follow-up.

Our results are in concordance with earlier reports of arrested bone loss by infliximab (108, 197) and indicate that early intensive treatment of the disease activity is beneficial to prevent bone loss.

The bone resorption markers, CTX-1 and 1CTP, decreased significantly from 3 to 12 months in the anti-TNF treated patients, p= 0.034 and 0.044, respectively. At 24 months, these decreases were still present, as a trend for CTX-1, p=0.07 and significantly for 1CTP, p =0.037. At this time-point also the bone synthesis marker, P1NP, had been significantly reduced, p = 0.041. There were no significant changes in any of the bone markers in treatment group A. The changes in markers of bone turnover were not significantly different between the treatment groups neither after 12 months nor after 24 months.

The changes in bone resorption markers are consistent with those found in an uncontrolled study, evaluating the effect of INF over 12 months in patients with established RA (31). The reduced bone turnover found in the anti-TNF treated patients favours the suggestion that TNFα has direct effects on inflammatory mediated bone loss. The reduction of 1CTP in the anti-TNF treated patients is of special interest. 1CTP is generated by MMPs that are associated with joint erosions in RA (36, 124). TNF blockade has been shown to reduce MMP levels and activity in patients with RA (29, 99). The reduction of 1CTP found here in the anti-TNF treated patients indicates a more pronounced inhibition of MMPs compared with the patients in treatment group A.

4.5 IGF-1 (I-IV)

In all papers, serum levels of IGF-1 as well as the age-standardized IGF-1 scores (IGFSD) were within the normal range. This more or less excludes inadequate energy- or protein intake as the cause of muscle wasting or bone loss as the sensitivity of circulating IGF-1 to nutrients, stability of its concentration and its short half-life makes it a useful marker of nutritional status and adequacy of nutritional rehabilitation (177).

In paper I, levels of IGFBP-1 was significantly elevated compared with a normal population, median (IQR) 58 (44-84) µg/L in women and 59 (24-76) µg/L in men, p<0.001 and 0.009, respectively. This is in line with the known stimulating effect of pro-inflammatory cytokines on IGFBP-1 production (53). As expected, we found a positive correlation between CRP and IGFBP-1 in this study (r=0.31, p=0.015). The

ratio IGF-1/IGFBP-1 was low, median (IQR) 2.0 (1.18-3.54) in women and 2.2 (1.1-9.2) in men, indicating a catabolic state (143).

In multiple regression analysis we found that IGF-1/IGFBP-1 was independently positively correlated with LBM, (beta=0.41, $p<0.001$).

Our data is in contrast to the findings of Rall et al (152) who found normal levels of IGFBP-1 and a trend for a lower level of IGF-1 in patients with RA. This discrepancy may be due to a higher disease activity in the present patients.

In the studies assessing the effects of low-dose prednisolone treatment, we found significantly higher IGF-1 levels in the prednisolone treated patients as well as significantly increased levels after 12 months GC treatment. Increase in circulating levels of IGF-1 during GC treatment has been shown earlier (46, 149, 163, 201). The mechanism behind the increase in IGF-1 is probably related to IGF-1 resistance with impaired IGF-1 signalling downstream the IGF-1 receptor. A suggested mechanism by which IGF-1 resistance leads to elevated circulating IGF-1 is decreased negative feedback regulation to the liver: Growth hormone releasing hormone from the hypothalamus stimulates pituitary GH release. GH stimulates IGF-1 production from the liver with subsequent stimulation of muscle growth directly and by inducing muscular IGF-1 production. Down regulation of local production of IGF-1 in muscle and consumption of liver IGF-1 decrease the negative feedback signal to the liver (163).

4.6 ADIPOKINES

4.6.1 Leptin (IV)

At baseline, leptin levels for lean patients were comparable with reference values. At baseline we found a positive correlation between leptin and BMI ($r=0.58$, $p<0.001$) and FM ($r=0.74$, $p<0.001$). This correlation was still present for all patients after 24 months. We did not find any correlation between leptin and variables of inflammation at baseline. After 24 months, there was a significant increase in leptin in both treatment groups, +3.2 (0.9-14.4) µg/L, $p=0.013$ in the DMARD combination group and +1.5 (0.4-7.0) µg/L, $p= 0.022$ in the anti-TNF treated group, although there was no significant difference in changes of leptin levels between the treatment groups.

The positive correlation between FM and leptin is in line with the fact that FM is the major determinant of plasma leptin concentrations (55). However, influences of inflammation on leptin levels have been evaluated earlier in RA with discordant results (6, 17, 145, 179). Furthermore, increased leptin synthesis in relation to inflammation has earlier mainly been described in conditions with highly activated immune system such in sepsis and septic chock (8, 55).

Anti-TNF therapy over 3 and 6 months has been reported not to alter leptin concentration (76, 144), which is in contrast to our findings. However, this difference could possibly be ascribed to different length of therapy as FM and BMI are the main determinants of circulating leptin and 3 and 6 months treatment is probably too short time to get a significant increase in BMI, which was reported to be stable in the former studies. The increase in leptin in treatment group A, despite lack of FM gain, may indicate a disruption between leptin levels and adiposity secondary to reduction of inflammation.

4.6.2 Adiponectin (IV)

At baseline, adiponectin levels were comparable with values from healthy individuals. Adiponectin increased significantly in both treatment groups after 12 months, +1.9 (0.4-3.5) mg/L, p=0.015 in treatment group A, and +2.3 (0.2-4.5) mg/L, p=0.037 in treatment group B, with no significant difference in changes between the treatment groups. Between 3 and 24 months there was a significant increase of adiponectin only in the treatment group A, +1.7 (0.1-3.2) mg/L, p=0.013 versus +2.4 (-0.7-5.4) mg/L, p=0.11 in treatment group B. The difference was, however, not significant between the treatment groups. At baseline we found no significant correlations between adiponectin and BMI, apolipoprotein A or variables of inflammation. In multivariate analysis we found a significant positive correlation between changes in FM from 3 to 24 months and adiponectin AUC ($r = 0.32$, $p = 0.047$).

The lack of significant correlations between adiponectin and BMI as well as the apolipoproteins is in contrast to earlier reports (89, 118). Further, the unexpected, positive correlation between changes in FM and adiponectin is opposite from the decreased levels of adiponectin seen in individuals with obesity (7). Reduction of adiponectin in obese individuals is suggested to, at least in part, be related to increase of TNFα, which is known to reduce adiponectin secretion (95).

Adiponectin improves insulin sensitivity (94) and has also been reported to have atheroprotective effects in RA patients (100). The increase of adiponectin found here, when disease activity decreased, might be one explanation for the reduced frequency of CVD reported in MTX treated RA patients (30) as well as during anti-TNF therapy (92).

5 GENERAL SUMMARY AND FUTURE PERSPECTIVES

The aims of this thesis were to study body composition and bone mineral density and the effects of inflammation and treatment with GCs and TNF- antagonists in patients with RA.

The most frequent changes in body composition were seen in RA patients with long-standing and active disease. It was especially obvious for loss of FFM, which was associated with disease activity, physical disability and low levels of IGF-1. In early RA, the proportion of patients with low FFM was much less and intensive treatment of the disease activity resulted in no further loss of FFM.

Increased FM, on the other hand, was rather common, both in longstanding and in early RA. Treatment with low-dose prednisolone as well as TNF-antagonists seemed to further aggravate the adiposity. Markers of inflammation did not correlate with FM. Physical disability, assessed as HAQ-score, was though correlated with FM. Physical disability is often associated with decreased physical activity, which may be one explanation for the FM gain. It is well known that GC treatment can result in weight gain and increased FM. The result from study II showed that also low-dose ($\leq$ 7.5 mg) prednisolone results in FM gain. Further, the result of study IV indicates that the fat gain in the anti-TNF treated patients was drug specific.

Osteoporosis was found in about one fourth of the RA patients with longstanding disease. Study IV, showed that early, intensive treatment of the disease activity can arrest bone loss. Results from study III indicate that addition of low-dose prednisolone can arrest bone loss in femoral neck. However, when low-dose prednisolone is used, it is important to supplement with vitamin D and calcium as well as consider treatment with bisphosphonates, especially in postmenopausal women.

Adipose tissue is interesting in many aspects. FM was increased in a large proportion of all RA patients. Further, this deterioration of body composition did not improve with treatment that decreased disease activity. The adipose tissue is clearly involved in the development of the metabolic syndrome and is a risk factor for CVD but it is also an important endocrine organ, producing a lot of adipokines.

In study IV, leptin and adiponectin increased in both treatment groups. There are contradictive reports of the role of leptin and adiponectin in RA. Considering leptin, most researchers agree that the main determinant of its serum concentration is the amount of FM and that it is not correlated with disease activity. It is thus not clear why leptin increased, despite stable weight and FM, when disease activity decreased in treatment group A. We believe that the increasing levels of adiponectin seen in both treatment groups in study IV were favorable in the context of CVD. However, there have been reports of both pro-inflammatory and anti-inflammatory effects of adiponectin. Thus, further studies of the effects of both leptin and adiponectin are warranting.

As decreasing disease activity does not seem to be enough to improve the detoriation of body composition and especially not the increased fat mass, it seems obvious that complementary approaches such as enhancing positive health behavior with increased

physical exercise and proper diet recommendations are important. After all, CVD is the most common cause of death in patients with RA and is important to prevent. To my knowledge, there are no intervention studies for CVD in RA including assessment of body composition and adipokines.

Treatment that decreased disease activity seemed to stop further loss of bone and FFM. However, for those patients that already have low FFM, to get an increase in skeletal muscle mass, high-intensive resistance training must be performed and the intake of protein must be adequate.

Strengths of the present work include the prospective, randomized design in study III and IV and that the work includes studies of both established and early RA. There are some limitations of this work. Both study III and IV were design to assess the effects of GCs and anti-TNF theraphy, respectively, on disease activity and joint damage and not body composition and BMD. The sample size in study IV was rather small and further differences between the treatment groups might be undetectable. The fact that the patients in study II partially were included at different time periods in the two treatment groups may have selected a control group with less severe disease, as during this time period the biologic agents were introduced and an exclusion criterion for the control group was current or previous treatment with TNFα blocking agents.

6 SVENSK SAMMANFATTNING

Reumatoid artrit (RA) eller ledgångsreumatism är en kronisk, inflammatorisk systemsjukdom som framför allt engagerar lederna. Förutom lederna kan även andra organ påverkas. Förändring i kroppssammansättningen av fett, muskler och skelett är också vanligt förekommande. Således har patienter med RA ofta en ökad fettmassa och en minskad muskelmassa. En ökad fettmassa är förenad med en ökad risk att utveckla typ 2 diabetes och hjärt-kärl sjukdomar. Skelettmuskulaturen är ett viktigt förråd av proteiner och utgör en viktig ”byggstensreserv” för kroppen. En minskad muskelmassa medför ökade svårigheter att klara av eventuella skador, infektioner eller sjukdomar. Tillsammans bidrar en ökad fettmassa med samtidig förlust av muskelmassa till en ökad sjuklighet och dödlighet hos patienter med RA. Den här avhandlingen syftade till att undersöka betydelsen av inflammation och effekten av olika medicinska behandlingar på förändringar i kroppssammansättning hos patienter med RA.

Alla patienter genomgick en dual X-ray absortiometry (DXA) eller ”bentäthetsundersökning” som även ger information om fett- och muskelmassa. Patienterna undersöktes också avseende aktivitet i ledsjukdomen samt svarade på frågor om hur de klarar aktiviteter i det dagliga livet, så kallat Health Assessment Questionnaire (HAQ). Vi analyserade också i blod olika markörer för benomsättning, den anabola peptiden IGF-1 samt cytokiner producerade från fettvävnaden i form av leptin och adiponectin. Två av studierna gjordes på patienter med RA som haft sin sjukdom sedan många år och två studier gjordes på patienter med nydebuterad RA, där vi följde patienterna under 2 år. I de två senare studierna studerade vi behandling med lågdos kortison samt TNF blockad.

I *delstudie I* fann vi att hälften av 60 RA-patienter i slutenvård med hög inflammatorisk aktivitet hade låg muskelmassa mätt som fettfri mass index (FFMI). Den låga muskelmassan var relaterad till en hög sjukdomsaktivitet, nedsatt fysisk funktion samt låga nivåer av IGF-1. Vi fann dessutom att 45% av dessa patienter hade en hög fettmassa mätt som fettmass index (FMI) och 80% hade en fettprocent överensstämmande med övervikt eller obesitas.

I *delstudie II* jämförde vi 50 patienter behandlade med lågdos kortison (7.5 mg) sedan minst 2 år med 50 patienter som inte behandlats med kortison. Vi fann att kortison behandlade patienter hade signifikant högre fettmassa än icke kortison behandlade patienter. Däremot var det ingen skillnad i bentäthet vare sig i ländrygg eller i höft. Nivån av markören för benbildning var jämförbar mellan behandlingsgrupperna. En bieffekt av kortison är annars en minskning av just benbildningen, en hämning som ofta uppträder framför allt under de första månaderna efter insatt behandling för att därefter stabiliseras.

I *delstudie III* har vi i en jämförande, kontrollerad studie följt totalt 150 nydebuterade RA patienter från olika centra i Sverige. Sjuttio av dem randomiserades till tillägg av lågdos kortison när de började med sin första sjukdomsmodifierande behandling (P-gruppen) medan 80 inte fick kortison tillägg (NoP-gruppen). I P-gruppen sjönk markören för benbildning snabbt medan den förblev oförändrad i NoP-gruppen. Benresorptionsmarkörerna sjönk också snabbt och mer uttalat än i NoP-gruppen. Bentätheten minskade i ländryggen i bägge behandlingsgrupperna under den 2 år långa behandlingsperioden. Däremot förblev bentätheten oförändrad i höften i P-gruppen till

skillnad mot i NoP-gruppen där den minskade. Resultaten talar för att kortison kunde bromsa upp den inflammationsorsakade benförlusten i höften tack vare att minskningen av bennedbrytningen dominerade över minskningen av benbildningen. Kortisonbehandlingen kunde däremot inte bromsa benförlusten i ryggen och ffa inte hos postmenopausala kvinnor som förlorade signifikant mer ben jämfört med postmenopausala kvinnor som inte fått kortison.

I *delstudie IV* har vi i en jämförande kontrollerad studie följt totalt 40 patienter med nydebuterad RA under 2 år. Patienterna randomiserades till studien ifall de svarat otillräckligt på behandling med Methotrexate efter 3 månader. De randomiserades då till tillägg av TNF-blockad (18 patienter) alternativt tillägg av sulfasalazin och antimalariamedel (22 patienter). Här fann vi att patienter som behandlades med TNF-blockad fick en ökad fettmassa, vilket inte var fallet i den andra behandlingsgruppen. Bägge grupperna hade likvärdig minskning av den inflammatoriska aktiviteten. Inte i någon av behandlingsgrupperna förekom någon benförlust eller minskning av muskelmassan. Leptin och adiponektin ökade i bägge behandlingsgrupperna. En ökning av adiponektin medför vanligtvis en bättre insulinkänslighet samt fördelaktiga effekter på blodkärlsväggen vilket kan bidra till en minskning av hjärtkärl sjukdom.

Sammanfattningsvis hade en stor andel av RA patienterna förändringar i sin kroppskomposition vilket kan öka risken för bland annat hjärtkärl sjuklighet. Kortisonbehandling liksom TNF blockad ökade fettmassan vilket man bör ta hänsyn till. Man bör även särskilt beakta risken för benskörhet hos postmenopausala kvinnor behandlade med kortison och ge förebyggande behandling med kalk och D-vitamin samt till vissa patienter även överväga läkemedel som hämmar bennedbrytningen.

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