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NUTRITIONAL STATUS, BODY COMPOSITION AND DIET IN PATIENTS WITH RHEUMATOID ARTHRITIS

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory disease with higher mortality rate than in the general population, which is largely attributed to cardiovascular disease (CVD). Another consequence of the inflammatory process is change in body composition with decreased muscle mass and increased fat mass. This condition has been named rheumatoid cachexia and is difficult to detect in clinical practice, as it is associated with little or no weight loss and with a maintained body mass index.

The aims of this thesis were to evaluate different diagnostic instruments for assessment of nutritional status and body composition in patients with RA and to study if the diet was associated with body composition derangement and dyslipidemia, especially antibodies against phosphorylcholine (anti- PC).

In- and out-ward RA patients at the Karolinska University Hospital and Södersjukhuset in Stockholm were included in the studies. They were assessed by anthropometric measures, dual-energy x-ray absorptiometry (DXA), bioelectrical impedance analyses (BIA) and nutritional questionnaires. Further blood samples and adipose tissue were analysed. Sixty-six patients were randomized to either a vegan diet free of gluten or a well-balanced non-vegan diet for 1 year and assessed as to disease activity and dyslipidemia.

Twelve per cent of the in-ward women, only one of the out-wards and none of the men had BMI<18.5, the cutoff value for malnutrition. Fifty-two percent of the in-ward women and 30% of the men were malnourished, according to fat free mass index (FFMI). Corresponding figures for the out-ward women and men were 26% and 21%, respectively. Reduced FFM was independently related to age, disease duration, erythrocyte sedimentation rate (ESR) and function trendwise. However, these patients also displayed central obesity in 57% of the women and in 89% of the men. About every fifth patient displayed concomitant low fat free mass (FFM) and elevated fat mass (FM), i.e. rheumatoid cachexia. These patients had significantly higher total cholesterol, LDL, and trendwise oxLDL as well as lower anti-PC, higher frequency of hypertension (69%) and metabolic syndrome (25%) than those without rheumatoid cachexia.

The anthropometrical measurements showed low sensitivity and high specificity for detecting malnutrition. Of the nutritional questionnaires Mini Nutritional Assessment (MNA) had the highest sensitivity but the specificity was low. There was a good relative agreement between DXA and BIA assessing body composition (FM, r^2=0.94, FFM, r^2=0.92; both $p<0.001$), but the limits of agreement were wide for each variable, i.e. for FM -3.3 to 7.8 kg; and for FFM -7.9 to 3.7 kg.

The patients reported a high dietary intake of saturated fat. However, patients with or without cachexia did not differ with respect to dietary fat intake or intake of mediterranean like diet. Patients on mediterranean like diet though had high levels of anti-PC. Gluten-free vegan diet induced lower low-density lipoprotein (LDL) levels and higher anti-PC IgM than a normal western diet ($p < 0.005$).

In conclusion, a large proportion of RA patients had reduced FFMI and central obesity. Rheumatoid cachexia was common and was not associated with dietary fat intake but with high LDL and low anti-PC levels. Gluten-free vegan diet in RA induced changes in serum lipids that are potentially atheroprotective and anti-inflammatory. Of the tested clinical evaluation tools, MNA might be used as a screening instrument. There was a good relative agreement between DXA and BIA, but the limits of agreement were wide, which may restrict the utility of BIA in clinical practice.

Key words: rheumatoid arthritis; rheumatoid cachexia; body composition; body mass index; nutritional status; nutritional questionnaires; dyslipidemia, oxLDL, anti-phosphorylcholine

LIST OF PUBLICATIONS

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

I. Elkan AC, Engvall IL, Tengstrand B, Cederholm T, Hafström I.

Malnutrition in women with rheumatoid arthritis is not revealed by clinical anthropometrical measurements or nutritional evaluation tools.

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II. Elkan AC, Engvall IL, Cederholm T, Hafström I.

Rheumatoid cachexia, central obesity and malnutrition in patients with low-active rheumatoid arthritis- feasibility of anthropometry, Mini Nutritional Assessment and body composition techniques.

Submitted.

III. Elkan AC, Sjöberg B, Kolsrud B, Ringertz B, Hafström I, Frostegård J.

Gluten-free vegan diet induces decreased LDL and oxidized LDL levels and raised atheroprotective natural antibodies against phosphorylcholine in patients with rheumatoid arthritis: a randomized study.

Arthritis Res Ther. 2008; 10(2):R34.

IV. Elkan A-C, Håkansson N, Frostegård J, Cederholm T, Hafström I.

Rheumatoid cachexia is associated with dyslipidemia and low levels of antibodies against phosphorylcholine but not with dietary fat in patients with rheumatoid arthritis: a cross sectional study.

Submitted.

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LIST OF ABBREVIATIONS

AA	Arachidonic acid
ACR	American College of Rheumatology
ADL	Activity of daily living
ALA	α-linolenic acid
AMC	Arm muscle circumference
Anti-PC	Antibodies against phosphorylcholine
BAPEN	British Association for Parenteral and Enteral Nutrition
BIA	Bioelectrical impedance analysis
BMI	Body mass index
CRP	C-reactive protein
CVD	Cardiovascular disease
DAS	Disease Activity Score
DAS28	Disease Activity Score including 28 joints
DHA	Docosahexaenoic acid
DMARD	Disease-modifying antirheumatic drug(s)
DXA	Dual-energy x-ray absorptiometry
EPA	Eicosapentaenoic acid
ESPEN	The European Society for Clinical Nutrition and Metabolism
EULAR	European League Against Rheumatism
FAs	Fatty acids
FFM	Fat free mass
FFMI	Fat free mass index
FFQ	Food-frequency questionnaire
FM	Fat mass
FMI	Fat mass index
HAQ	The Stanford Health Assessment Questionnaire
HDL	High-density lipoprotein
IDF	International Diabetes Federation
IL-6	Interleukin 6
LA	Linoleic acid
LDL	Low- density lipoprotein
MAC	Mid arm circumference
MetS	Metabolic syndrome
MNA	Mini Nutritional Assessment
MUFAs	Monounsaturated fatty acids
MUST	Malnutrition Universal Screening Tool
NRS-2002	Nutritional Risk Screening
NSAID	Non-steroidal anti-inflammatory drug(s)
OxLDL	Oxidized low-density lipoprotein
PUFAs	Polyunsaturated fatty acids
RA	Rheumatoid arthritis
RF	Rheumatoid factor
SFAs	Saturated fatty acids
SGA	Subjective Global Assessment
TNF- α	Tumor necrosis factor alfa
TSF	Triceps skin fold thickness
WC	Waist circumference
WHO	World health organization

1 BACKGROUND

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of unknown cause. It affects about 0.5% of the adult population and is 2-3 times more common in women than in men. Any joint may be affected but the disease is most commonly localized in hands, feet and wrists. It is a painful condition and can cause severe disability and ultimately affects a person's ability to carry out everyday tasks. Other symptoms include fatigue, malaise, and morning stiffness. Extra-articular involvement of organs such as the skin, heart, lungs, and eyes can be significant. Thus RA may lead to considerable morbidity and is also associated with increased mortality.

The excess mortality may be apparent within the first few years of disease and increases with RA disease duration [81]. This increased mortality is largely attributed to cardiovascular disease (CVD) [69, 110].

1.1 DIAGNOSIS AND ASSESSMENT OF DISEASE ACTIVITY AND FUNCTION IN RA

The diagnosis of RA is often based upon the classification criteria (table 1) created by the American College of Rheumatology (ACR). These criteria were developed on patients with established disease [7].

Table 1. Criteria for the classification of rheumatoid arthritis. A patient shall be said to have rheumatoid arthritis if at least 4 of these 7 criteria are present. The first four criteria must have been present for at least 6 weeks. Adapted from Arnett 1988.

Criterion	Definition
Morning stiffness	Morning stiffness in and around the joints, lasting at least 1 hour before maximal improvement.
Arthritis of 3 or more joint areas	At least 3 joint areas simultaneously have had soft tissue swelling or fluid.*
Arthritis of hand joints	At least 1 area swollen in a wrist, MCP, or PIP joint
Symmetric arthritis	Simultaneous involvement of the same joint areas on both sides of the body.
Rheumatoid nodules	Subcutaneous nodules, over bony prominences, or extensor surfaces, or in juxta-articular regions.
Serum rheumatoid factor	Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in <5% of normal control subjects.
Radiographic changes	Radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs.

*The 14 possible areas are right or left proximal interphalangeal (PIP), metacarpophalangeal (MCP), wrist, elbow, knee, ankle, and metatarsophalangeal (MTP) joints.

Disease activity is often measured by a composite score, the Disease Activity Score (DAS) [124]. This score includes the patient's global assessment of general health, number of swollen and tender joints as well as the erythrocyte sedimentation rate (ESR). When restricting the number of assessed joints to 28, the DAS28 is calculated [93]. A DAS28 value >5.1 is regarded as high disease activity, ≤3.2 is regarded as low activity and <2.6 is regarded as disease remission. In this context it should however be remembered that women score higher than men, mainly due to higher pain perception despite similar disease severity [4].

DAS28 has during the last decade been increasingly used to make decisions of treatment strategies as well as to monitor treatment outcome in RA. To assess response to treatment the European League Against Rheumatism (EULAR) has defined response criteria consisting of a combination of a significant reduction of baseline disease activity and the level of disease activity attained. Good response is defined as a decrease of DAS28 >1.2 in combination with an achieved disease activity of ≤3.2. Non-response is defined as a decrease of DAS28 ≤0.6, or a decrease between >0.6 and ≤1.2 with an attained DAS28 >5.1. Any other changes are regarded as moderate responses [125].

Another measurement of treatment response is defined by the ACR. The ACR20 response requires at least a 20% improvement in both the number of swollen and tender joints, as well as a 20% improvement in three of the following five variables: ESR, Health Assessment Questionnaire (HAQ) score, the physician's and the patient's global assessments of disease activity and the patient's perceived pain [32]. Correspondingly, the ACR50 and the ACR70 responses require a 50% and a 70% improvement, respectively.

Functional status is mostly measured by The Stanford Health Assessment Questionnaire (HAQ), which is a self-reported instrument evaluating capacity to perform activities of daily living. It consists of 20 items in eight categories of activities of daily living (ADL): Dressing and grooming, Arising, Eating, Walking, Hygiene, Reach, Grip, and Some Additional Common Daily items. The response score alternatives for each of the 20 items are: "without any difficulty" (= 0), "with some difficulty" (= 1), "with much difficulty" or "with use of assistive devices" (= 2), and "unable to do" (= 3). The highest item score in a given category determines the score of that category. The total HAQ score ranges from 0 -3, based on the sum of the scores of each category, divided by the number of categories responded to [33]. A validated Swedish version of the Stanford Health Assessment Questionnaire (HAQ) has been developed by Ekdahl et al. [28].

Increased HAQ score is a consequence of both inflammation and joint destruction [45, 104]. Thus, during the first years of disease inflammation is considered to be the main contributor to the level of disability, whereas later on joint damage has a greater impact.

1.2 RA AND CARDIOVASCULAR DISEASE (CVD)

RA is associated with increased cardiovascular morbidity and mortality. This appears to be predominantly due to ischemic causes, such as myocardial infarction, stroke and congestive heart failure. The high prevalence of cardiac ischemia in RA is thought to be due to accelerated development of atherosclerosis. There are two main reasons for this, which might be interrelated: the systemic inflammatory load, characteristic of RA, and the accumulation in RA of classical risk factors for coronary heart disease, e.g. smoking and the metabolic syndrome [80, 111]. Growing evidence points to that inflammation in RA is associated with a worsening of the lipid profile [17, 89], present already early in the disease [39]

1.3 RA AND DYSLIPIDEMIA

Dyslipidemia in RA is mainly presented by low concentration of high-density lipoprotein (HDL), associated with an unfavourable cardiovascular risk. Total cholesterol and HDL levels in RA are inversely associated with the acute phase response, regardless of whether patients are treated with antirheumatic drugs or not [39]. Furthermore, patients with RA have increased levels of oxidized low-density lipoprotein (oxLDL) in serum compared with healthy subjects, which may contribute to the increased risk of cardiovascular disease in this patient group [57, 67] as LDL oxidation probably has an important role in the pathogenesis of atherosclerosis [111].

1.3.1 Antibodies against phosphorylcholine (anti-PC)

Exposed on oxLDL is phosphorylcholine (PC), which is a major ligand for inflammatory phospholipids like platelet-activating factor (PAF). PC is a target of natural antibodies against PC (anti-PC), which by binding to the surface of oxLDL blocks uptake of oxLDL by macrophages and facilitates its clearance [97]. It has been demonstrated that anti-PC of IgM (anti-PC IgM) subclass is a protective factor for atherosclerosis in patients with established hypertension [113]. However, in RA anti-PC has not been studied in relation to CVD.

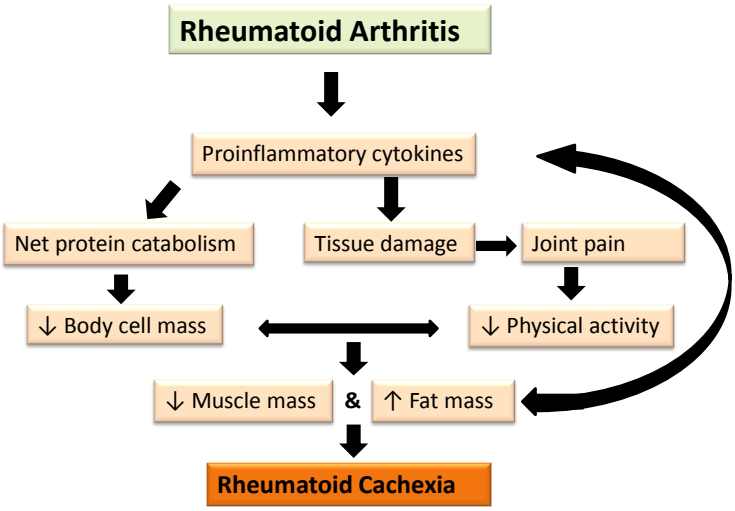
1.4 BODY COMPOSITION AND NUTRITIONAL STATUS

1.4.1 Body composition

The RA disease is often associated with change in body composition. Thus the patients have reduced fat free mass (FFM), of which muscle mass is the largest component [100, 121], often in combination with increased body fat mass (FM). Consequently they have little or no weight loss and a maintained body mass index (BMI) [94, 129]. This condition has been named “rheumatoid cachexia” [98, 122]. Besides to muscle weakness and disability the rheumatoid cachexia contributes to increased risk of infections and is believed to accelerate morbidity and mortality in RA [94, 122],

Rheumatoid cachexia has been described in up to two-thirds of RA patients and is suggested to be caused by cytokine-driven hyper metabolism [70, 99, 121, 122], figure 1. However it has also been found in patients with good disease control [121]. The frequency of occurrence of rheumatoid cachexia depends upon what degree of reduction of muscle mass is considered to be significant. Obesity in combination with decreased FFM may potentiate the negative effects of each other and thus result in higher risk of physical disability, morbidity and mortality.

Figure 1. The actions of proinflammatory cytokines in RA.



RA is characterized by excess production of the inflammatory cytokines, which shift protein metabolism toward net catabolism, mediate joint and bone destruction, and cause joint pain and stiffness. This causes loss of body cell mass, predominantly skeletal muscle mass, and reduced physical activity, which reinforce each other and lead to further losses of skeletal muscle mass and predispose to fat gain. Fat gain increases circulating inflammatory cytokines (produced by adipocytes). This adds to the negative cycle of muscle loss and fat gain, and causes rheumatoid cachexia.

1.4.1.1 Definition of cachexia

Cachexia is referred to as wasting disease, malnutrition, or hyper catabolism. The disease is encountered in many malignant and nonmalignant chronic, ultimately fatal, illnesses. The loss of skeletal muscle mass is due to a combination of reduced protein synthesis and increased protein degradation. Although reduced protein synthesis plays a role, protein degradation is the major cause of loss of skeletal muscle mass [116].

Cachexia is also viewed as cytokine-associated wasting of protein and energy stores. Systemic inflammation mediated through cell injury or activation of the immune system triggers an acute inflammatory response. Subjects with cachexia lose roughly equal amounts of fat and fat-free mass, while maintaining extra cellular water and intracellular potassium. The loss of fat-free mass is mainly from the skeletal muscle [116].

There is a debate regarding the definition of cachexia, which is still ongoing. The European Society for Clinical Nutrition and Metabolism (ESPEN) has defined cachexia as “a documented non-intentional weight loss of more than 6% in the previous 6 months, accompanied by catabolic conditions and resistance to increased substrate intake” [66].

Recently a further definition has been published in combination with a proposal to assess cachexia in clinical practice [31]. “Cachexia is a complex metabolic syndrome associated with underlying illness and characterized by loss of muscle with or without loss of fat mass. The prominent clinical feature of cachexia is weight loss in adults (corrected for fluid retention) or growth failure in children (excluding endocrine disorders). Anorexia, inflammation, insulin resistance and increased muscle protein breakdown are frequently associated with cachexia. Cachexia is distinct from starvation, age-related loss of muscle mass, primary depression, malabsorption and hyperthyroidism and is associated with increased morbidity".

The proposed definitions of cachexia do not fit the body composition changes occurring in RA patients, since these patients don’t lose weight and they don’t lose fat mass. Therefore the concept of rheumatoid cachexia has been established to separate this condition from cachexia in other diseases.

1.4.1.2 Definition of sarcopenia

The term sarcopenia is primarily used to describe the process of age-related muscle loss [66, 78], although it is sometime used also in younger ages to describe isolated loss of muscle mass [84]. Sarcopenia is, as rheumatoid cachexia, associated with maintained or increased fat mass, i.e. stable weight or gain of weight [9, 116, 138]. Sarcopenia is mediated by a number of factors, including age-related decline in hormones, degeneration of muscle innervations, genetic factors, reduced activity levels or coexisting disability.

1.4.1 Nutritional status

There are only a few studies exploring the nutritional status among patients with RA. Due to different methods and varying populations there is a large discrepancy between studies of prevalence of malnutrition in these patients, ranging from 26% to 71% [22, 51, 52, 79, 95]. The true prevalence of malnutrition further depends on which criterion that is used to define this condition. Additionally there are no validated assessment tools to detect malnutrition in RA patients.

In spite of the limitations mentioned above malnutrition in RA has been considered to be associated with higher rates of complications, increased nosocomial infections, higher hospital costs, higher mortality and longer length of hospital stay [21, 24, 75, 96, 102].

1.4.1.1 Definition of malnutrition

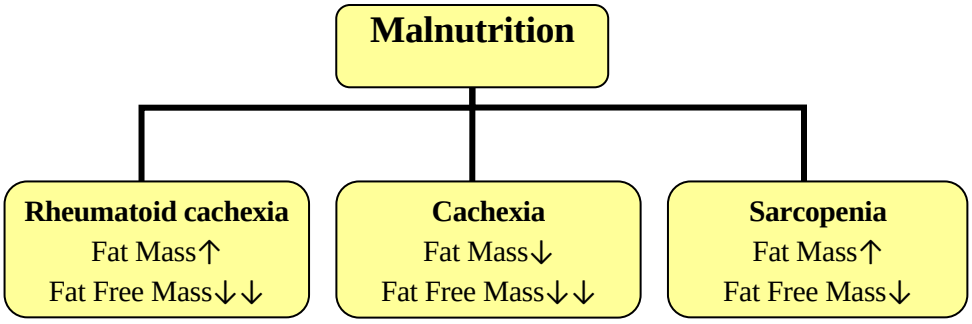
There is no consensus as to the definition of malnutrition. WHO's definition is "the cellular imbalance between supply of nutrients and energy and the body's demand for them to ensure growth, maintenance and specific functions" [130], which is a broad definition that includes both supply and demand.

ESPEN has defined malnutrition as "a state of nutrition in which a deficiency or excess (or imbalance) of energy, protein, and other nutrients causes measurable adverse effects on tissue/body form (body shape, size and composition) and function, and clinical outcome" [66].

Yet another definition have been proposed recently for malnutrition as ''a subacute or chronic state of nutrition in which a combination of varying degrees of over- or under nutrition and inflammatory activity has led to a change in body composition and diminished function'' [108].

Not until the nutrition world agrees upon what malnutrition means, it will become possible to reach consensus how to assess nutritional status and how to diagnose this condition. Despite the fact that there is no agreement on the definition of malnutrition, it appears to be general agreement that cachexia and sarcopenia belong to the malnutrition syndromes (figure 2).

Figure 2. Displays a scheme where malnutrition serves as an "umbrella" for other malnutrition syndromes.



1.4.1 Body composition assessment

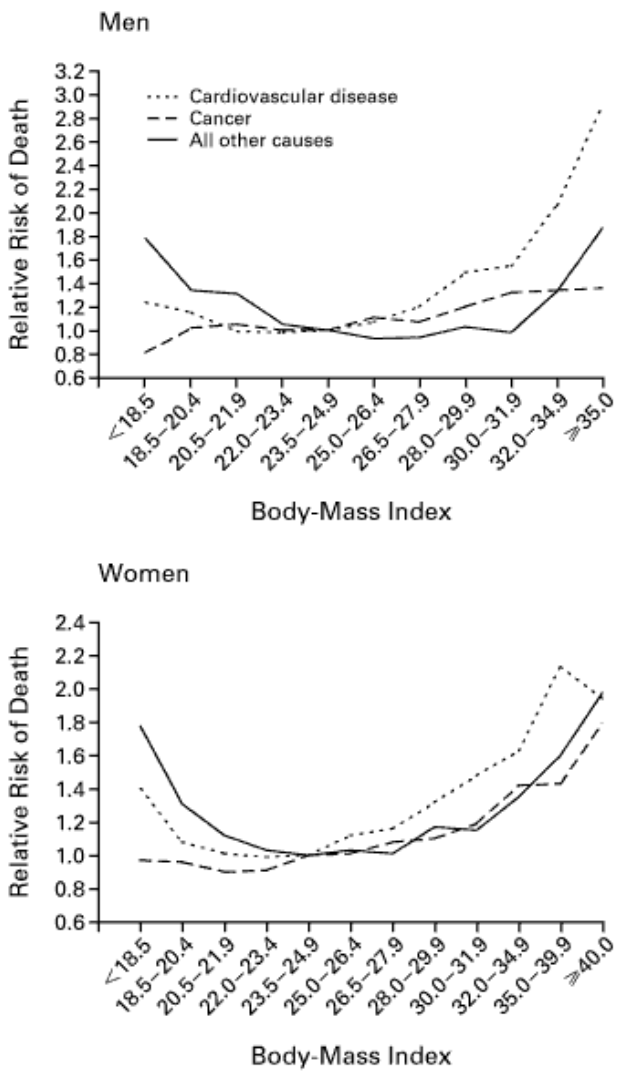
Several diagnostic methods have been used to assess body composition. Some of them are: anthropometrical measures, whole-body dual-energy x-ray absorptiometry (DXA) and bioelectrical impedance analysis (BIA). These two last methods measure FFM, which includes muscle, organ tissue and skin as well as bone, and FM separately. The possibility to determine FFM and FM separately gives the opportunity to detect cachexia and malnutrition in chronically ill patients.

1.4.1.1 Body mass index (BMI)

BMI has traditionally been used to identify individuals who are overweight or obese. BMI is calculated from weight/height² (kg/m²). BMI values below 18.5 kg/m^2 corresponds to underweight/malnourishment, between 18.5 - 24.9 to normal, 25-29.9 to overweight and values greater than 30 to obesity [131].

In a large, prospective study, the lowest rates of death from all causes were found at BMI values between 23.5 and 24.9 in men and 22.0 and 23.4 in women. Death rates increased throughout the range of moderate and severe overweight in all age groups and for all categories of causes of death. Also low BMI has been found to be associated with an increased morbidity, especially respiratory and cerebrovascular diseases [19].

Figure 3. Multivariate relative risk of death from cardiovascular disease, cancer and all other causes among men and women [19]



In patients with RA low BMI (<20 kg/m²) is an important predictor of increased cardiovascular mortality. Low BMI at time for RA diagnosis is associated with a 3-fold increased risk of cardiovascular death compared with that in non-RA subjects with normal BMI. The risk associated with low BMI persists irrespective of whether the patients maintain low BMI or achieve a normal BMI during the disease course. Furthermore, if normal BMI at the time of RA diagnosis, patients getting low BMI during the disease course also have a significantly increased risk of cardiovascular death [63].

Compared with non-RA subjects BMI has a paradoxical association with mortality in RA patients. Thus patients with high BMI have lower mortality than thinner patients. This relation is due in part to co-morbidity in the thinner patients. The importance of body mass on survival seems thus to be modified by the level of systemic inflammation [30].

1.4.1.2 Waist circumference (WC)

WC was developed initially as a simple measure and a potentially better indicator of health risk than BMI to be use in health promotion. WC alone gives a good prediction of visceral and total fat. People with a large waist are many times more at risk of bad health, including features of metabolic syndrome (such as diabetes, hypertension and dyslipidemia) as well as shortness of breath and poor quality of life. These increased risks also apply to people whose BMI is normal but who have a large waist. However, BMI and waist circumference are colinear, so combining the two measures adds relatively little to risk prediction [50].

According to the International Diabetes Federation (IDF) a waist circumference less than 94 cm in men and 80 cm in women would be low risk for type 2 diabetes; an intermediate risk is indicated by 94–101.9 cm in men and 80–87.9 cm in women. A value above 102 cm in men and 88 cm in women indicate a high risk for type 2 diabetes, coronary heart disease or hypertension [6].

1.4.1.3 Triceps skin fold thickness (TSF)

TSF reflects calorie reserves stored in form of fat and is measured over the triceps at the midpoint between the acromion and olecranon using a Harpenden Caliper. The size of the skin fold in an individual can vary according to duration and level of compression [120]. Measuring TSF is thus problematic and measurement error makes interpretation difficult. The cut-off values for malnutrition, used in this thesis, are defined as TSF below the 10th percentile of a Swedish reference population. That corresponds to TSF below 13–16 mm for women and 5–7 mm for men, age dependent [115].

1.4.1.4 Arm muscle circumference (AMC) and mid arm circumference (MAC)

AMC is an indicator of muscle protein stores and is calculated from MAC and TSF, using the formula AMC (cm) = MAC (cm) – 3.14 x TSF (cm). MAC is measured at the midpoint between the acromion and olecranon. The cut-off values for malnutrition, used in this thesis, are defined as AMC values below the 10th percentile of a Swedish reference population. That corresponds to AMC below 19 cm for women and 22–23 cm for men, age dependent [115]. In RA patients AMC and MAC may be influenced by local disease in the elbow or shoulder [76].

1.4.1.5 Whole-body dual-energy x-ray absorptiometry (DXA)

DXA is based on a three-compartment model that divides the body into bone mineral, lean body mass and FM. This technique assumes that bone mineral content is directly proportional to the amount of photon energy absorbed by the bone being studied. DXA uses a whole body scanner that has two low doses x-rays at different sources that read bone and soft tissue mass simultaneously. The sources are mounted beneath a table with a detector overhead. The scanner passes across a person's reclining body with data collected at 0.5 cm intervals. A scan takes between 10-20 minutes. The use of DXA is suggested to be a fairly exact method to measure FFM and FM. It is often used as reference method [64]. The DXA method is considered sensitive to changes in body composition following weight gain or loss and changes related to ageing [41, 92, 134]. It is however expensive and time-consuming why it is not a method that can be routinely used in clinical practice.

1.4.1.6 Bioelectrical impedance analysis (BIA)

BIA is a simple, inexpensive and non-invasive method of assessing body composition. It is based on the underlying principle that resistance or impedance to the flow of an electrical current through the body is dependent on three variables: the length of the conductive path, the volume of the conductive material, and the resistivity of the conductive material. In humans, only body water, with its dissolved electrolytes, will conduct a current. Hence, using the assumption that the resistivity of the conductive material is constant and calculating the length of the conductive path from an individual's height, total body water (TBW) can be estimated by measuring impedance to the flow of a small current. Assuming that TBW constitutes a fixed percentage of lean mass (usually 73%), body composition can be estimated. Specific prediction equations have been developed to evaluate body composition from height, weight, and impedance [64].

1.4.1.7 Cut off values for low fat free mass or skeletal muscle mass

Several proposals have been addressed how to define reduced muscle mass and FFM. Most of these express muscle mass and FFM in relation to height, which facilitates the interpretation of body composition variables regardless of height. The primary limitation of those definitions is that it is still unknown what loss that is clinically important.

The concept of fat free mass index (FFMI, kg/height^2) was proposed by Kyle et al., who defined subjects having FFMI values under the 10th percentile of a large reference population as nutritionally depleted [65]. In that study the BMI cut-off points of 18.5, 20 and 25 kg/m^2 corresponded to FFMI percentiles P10, P25 and P75 of healthy adults, respectively.

A widely used definition of decreased skeletal muscle mass was proposed by Baumgartner et al. [10]; they accounted body size by dividing appendicular skeletal muscle mass (ASM), as assessed by DXA, by height squared (kg/height^2), thus obtaining a relative skeletal muscle index. Sarcopenia was defined as ASM/h^2 being less than two standard deviations below the mean of a young reference group.

Janssen et al. [56] proposed the use of percentage skeletal muscle index (SMI%, total muscle mass/body mass x100) and used BIA to evaluate muscle mass. The prevalence of reduced muscle mass is classified in relation to young adults. This index has the advantage of being able to account for body mass with a relative simple method, such as BIA. Sarcopenia was defined as SMI within -1 to -2 standard deviations of young adult values. This group [55] has also proposed skeletal muscle cut off points for identifying elevated physical disability risk in older adults.

1.4.1 Nutritional assessment

Numerous validated assessment and screening tools are available. Among these the Mini Nutritional Assessment® (MNA) and the Subjective Global Assessment (SGA), Malnutrition Universal Screening Tool (MUST) and Nutritional Risk Screening (NRS-2002), can be found.

1.4.1.1 The Mini Nutritional Assessment (MNA)

The MNA is a nutritional assessment tool that has been designed and validated to provide a single, rapid assessment of nutritional status in elderly patients [44, 128]. It has been translated into several languages and validated in many clinics around the world. It is composed of a dietary questionnaire (questions related to number of meals, food and fluid intake and autonomy of feeding), subjective assessment (self-perception of health and nutrition), global assessment (question related to life-style, medication and morbidity) and anthropometrical measurements (weight, height, and weight loss). MNA classifies individuals with adequate nutritional status (MNA $\geq$24), with risk for malnutrition (MNA 17-23.5) and with malnutrition (MNA <17).

1.4.1.2 The Subjective Global Assessment tool (SGA)

SGA consists of two parts, which take into account both the patient history and a clinical examination [25]. The patient history consists of questions concerning: height; weight current, 6 months and 12 months ago as well as changes in weight during the past 2 weeks; level of food intake the previous month compared with normal intake; problems preventing adequate intake the past 2 weeks, i.e. nausea and diarrhoea and functional capacity the last month.

The remaining questions, posed to the doctor or the nurse, deals with diagnosis, tumour stage, metabolic demand (influence of disease and/or treatment), and loss of subcutaneous fat, muscle wasting, oedema and ascites.

The instrument has been translated into Swedish and tested for validity and reliability in patients with gastrointestinal and geriatric diseases [27, 119]. SGA classifies patients into SGA A (well nourished), SGA B (moderately/suspected of being malnourished) and SGA C (severely malnourished).

1.4.1.3 The Malnutrition Universal Screening Tool (MUST)

The MUST was developed by the British Association for Parenteral and Enteral Nutrition (BAPEN) [112, 127]. The MUST includes three clinical variables and rates each variable as 0, 1 or 2 as follows: BMI>20 kg/m² = 0, 18.5-20 kg/m² = 1 and <18.5 kg/m² = 2; weight loss <5% = 0, 5-10% = 1 and >10% = 2; acute disease: absent = 0 and if present = 2. Overall risk of malnutrition is established as follows: 0 = low risk; 1 = medium risk; 2 = high risk.

1.4.1.4 The Nutritional Risk Screening tool 2002 (NRS-2002)

The NRS-2002 is endorsed by ESPEN [61, 62]. The NRS-2002 consists of a nutritional score and a severity of disease score and an age adjustment for patients > 70 years (+1). Nutritional score: weight loss >5% in 3 months or food intake below 50-75% in preceding week =1, weight loss >5% in 2 months or BMI 18,5-20,5 kg/m² and impaired general condition or food intake 25-50% in preceding week = 2, weight loss >5% in 1 month or 15% in 3 months or BMI <18,5 kg/m² and impaired general condition or food intake 0-25% in preceding week = 3. Severity of disease score: hip fracture, chronic patients with acute complications = 1, major abdominal surgery, stroke, severe pneumonia or haematological malignancies = 2, head injury, bone marrow transplantation, intensive care patients with APACHE score >10 = 3. NRS-2002 score is the total of the nutritional score, severity of disease score and age adjustment. Patients are classified at no risk = 0, low risk = 0-1, medium risk 3-4 and high risk = >5.

1.5 RA AND DIET

Even though diet therapy is generally not a part of the clinical practice of RA, many RA patients consider changing their diet. That is because they believe that there is a link between diet and the disease, whereas rheumatologists often are skeptical. It has been reported that 33% to 75% of RA patients believe that food plays an important role for their symptom severity, and 20% to 50% have tried dietary manipulation in an attempt to relieve symptoms [72, 101]. Besides fasting, various vegetarian diets including the most pure one, the vegan diet free of gluten are particularly popular. Additionally, the mediterranean diet has been increasingly used. The scientific basis for a role of dietary therapy in RA has grown in the last few years, although there is still no consensus on the nature of the optimum diet.

Fasting for about one week has been found to have favorable clinical and laboratory effects in RA patients, though of short duration [46, 107]. One suggested mechanism for this effect is altered fatty acid composition of membrane phospholipids with reduced release of leukotriene B4 [46].

Dietary elimination combined with a vegan diet free of gluten for three months followed by lactovegetarian food for 9 months resulted in significant improvement in signs and symptoms of RA as compared to a control, non-vegetarian diet. Among the diet responders, improvements persisted for an additional year after the study completion, though the diet generally focused on avoidance of foods that worsened symptoms [58-60]. The beneficial effect could not be explained by antibody activity against food antigens, or changes in concentrations of prostaglandin and leukotriene precursors.

A controlled study of a vegan, gluten-free diet for one year showed improved clinical status, lowered inflammatory markers and significantly reduced antibodies to gliadin and lacto globulin in the diet responders. The positive effect of the vegan diet in this subgroup was suggested to be due to diminished immune response to exogenous food antigens [47].

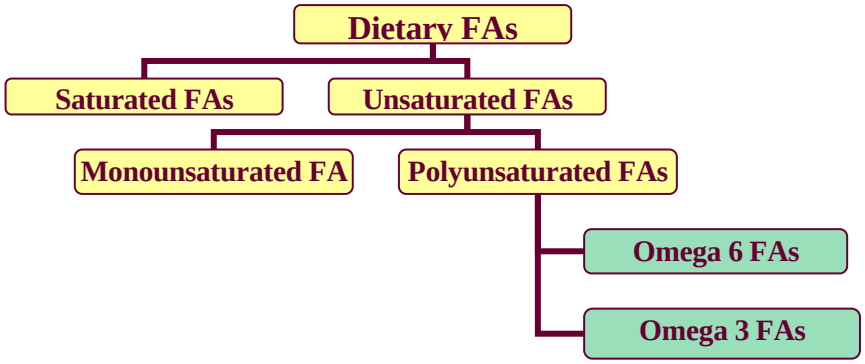
Theorizing that the anti-inflammatory nature of the diet would be beneficial in RA, Sköldstam et al. [106] designed a three-month study comparing the cretan mediterranean diet versus an ordinary western diet in patients with active RA. The cretan diet was relatively rich in fish, olive oil and canola (rapeseed) oil, whereas the intake of other fats and of red meat was low. In the experimental group a reduction in inflammatory activity was obtained and physical function and quality of life improved. Interestingly, those in the diet group that showed a moderate or better clinical improvement (diet responders) reported a higher intake of n-3 fatty acids and a lower ratio of n-6 to n-3 fatty acids compared with the patients with minor or no improvement. Also the fatty acid profile in serum phospholipids differed between the diet responders and the diet non-responders, consisting of increased relative amounts of the n-3 fatty acids EPA and DHA, and of the total percentage of n-3 fatty acids in responders [48]. These changes in lipids further highlight the association of mediterranean diet with reduced cardiovascular disease [109], a frequent co-morbidity in RA.

Dietary intake, in terms of energy and protein, has been reported to be adequate among patients with RA, why inadequate intake has been suggested not to contribute significantly to rheumatoid cachexia [43, 94, 99]. However, inadequate nutrient intake has also been reported [8, 77, 123].

1.5.1 RA and fatty acids (FAs)

There are three major groups of FAs; saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and n-3 and n-6 polyunsaturated fatty acids (PUFAs) figure 4. The two dietary PUFAs, linoleic acid (LA; 18:2 n-6) and α-linolenic acid (ALA; 18:3 n-3) cannot be endogenously synthesized in humans and are thus essential and must be provided with the diet. LA and ALA are precursors for eicosanoids, prostaglandins and leukotrienes.

Figure 4. The family of dietary fatty acids (FAs)



The impact of dietary fatty acids on rheumatoid inflammation has been investigated in a number of studies. The FAs most thoroughly studied in relation to RA, are the n-3 FAs (omega 3 FAs) especially eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). These PUFAs are found predominantly in fatty fish, such as herring, mackerel and salmon, and in more concentrated amounts in fish oil. Several randomized controlled studies have shown that supplementation with fish oil in addition to ordinary pharmacologic treatment results in beneficial effects on RA symptoms, such as decreased number of tender and swollen joints as well as a NSAID-sparing effect [36, 42].

There are several mechanisms by which increase of dietary n-3 FAs may reduce inflammation. Mostly there is a balance between n-3 and n-6 FAs (omega 6 FAs) intake, which means that when n-3 intake is increased n-6 is decreased and contrariwise. The dietary FA intake affects the composition of FAs in the cell membrane phospholipids. Individuals consuming a typical western diet have a high intake of n-6 FA and consequently have a high proportion of the n-6 PUFA arachidonic acid (AA; 20:4n-6) in their cell membranes. AA may contribute to the inflammatory processes by acting as a precursor to eicosanoids known to be proinflammatory in RA. Instead, n-3 PUFAs may act as anti-inflammatory agents by competing with AA for incorporation into inflammatory cell membranes and for metabolism by enzymes of eicosanoids

synthesis [18]. Thus, it is possible that greater efficacy of n-3 PUFAs may be achieved in RA by simultaneously actively decrease n-6 PUFA intake [2].

Consumption of SFAs reduces the anti-inflammatory potential of HDL and impairs arterial endothelial function. In contrast, the anti-inflammatory activity of HDL improves after consumption of PUFA. These findings highlight novel mechanisms by which different dietary FA may influence key atherogenic processes [85].

1.5.1 Dietary assessment

There are a number of methods that can be used alone or in combination to monitor dietary intake. The most common tools used to assess dietary intake are diet recall (24-48 hours recall), diet records (3-7 days food records), food-frequency questionnaire (FFQ), diet history interview and weighed diet records. These methods, except FFQ, are used to assess the current diet. FFQ is a retrospective method, which is used to assess the habitual dietary intake during the past year.

FFQ is widely used to assess long-term dietary consumption. FFQ was designed to classify individuals according to levels of average daily intake of selected nutrients from food and dietary supplements [74]. The questionnaire contains questions about how often 88 food items are used over the past year, with 9 possible frequency categories in increasing order from never or almost never to 3 times per day. There are also open-ended questions about the quantity of food items eaten daily by most Swedes, e.g., milk, bread, coffee, and cheese [74].

The accuracy of FFQ has been proven for in healthy individuals in terms of long-term dietary fat consumption as that corresponds to fatty acid composition in adipose tissue [38, 54, 118, 137]. However, using this technique, it is found that respondents often under-report consumption, a practice more common amongst those who are overweight than those who are lean [53].

1.5.2 Fatty acids in adipose tissue as biomarkers

By using biological markers, i.e. markers in biological specimens that reflect dietary intake, an objective measurement of the dietary intake may be obtained. The FA composition of adipose tissue has been considered a gold standard for the representation of dietary FAs, due to the slow turnover time of FAs in adipose tissue [53].

Optimal FA biomarkers include those that cannot be endogenously synthesized, such as PUFAs (n-3 and n-6) and odd-numbered SFAs (15:0, 17:0). Other saturated and monounsaturated fatty acids are not considered good biomarkers of intake because they are also endogenously synthesized [11].

In general, the FA composition of subcutaneous adipose tissue taken from sites such as the buttock and abdomen appears to be similar. Few studies have compared the adipose tissue fatty acid composition from these two sites. They report small but significant differences in the proportions of SFAs and MUFAs present, with the abdomen having higher SFAs and lower MUFAs than the buttock [53], whereas there were no differences regarding PUFAs between sites [37].

2 AIMS

The main objectives of this thesis were to:

- evaluate which of the method/methods applied for nutritional- and body composition assessments that have the best discriminating power, and could with ease be used routinely in medical care, to identify RA patients with malnutrition and body composition derangement. *Paper I and II*

- study in what proportion in- and out-ward patients with RA suffer from malnutrition and body composition derangement. *Paper I and II*

- study if a vegan diet can affect the serum lipids and anti-PC in RA. *Paper III*

- study, which impact the diet content has on body composition, serum lipids and anti-PC in RA patients. *Paper IV*

- study if rheumatoid cachexia relates to cardiovascular risk factors in RA patients. *Paper IV*

3 PATIENTS AND METHODS

3.1 PATIENTS

In *paper I*, 60 consecutive patients admitted to the in-patient ward at the Rheumatology Department, Karolinska University Hospital Huddinge were included into the study, table 2. The patients should be aged 18– 80 years, have a diagnosis of RA and have disease duration of ≥2 years. The included patients did not have any severe comorbidities. They were mainly admitted for considering new antirheumatic medication. A total of 104 RA patients could not be included as they met one or more of the following exclusion criteria:

- current malignancy
- severe cardiac insufficiency according to the New York Heart Association classification (NYHA)>3
- severe renal failure (glomerulus's filtration rate <20 ml/min)
- chronic obstructive lung disease with emphysema
- earlier gastric ulcer or intestinal surgery
- known eating disorder
- glucocorticoid injections within 2 weeks

Table 2. Clinical characteristics of the 60 in-ward RA patients

	Women, N=50	**Men, N=10**
Age (years) *	65.5 (60.0-75.0)	60.5 (55.0-67.0)
Disease duration (years) *	13.0 (9.0-23.0)	15.5 (6.0-24.0)
DAS28	5.7 (5.3-6.1)	4.5 (4.0-4.8)
HAQ score	1.9 (1.7-2.1)	1.4 (0.6-2.1)

*= median. Data is presented as mean (CI) for normally distributed variables, and as median (inter-quartile range) for non-parametric variables. N=number

In *paper II* and *IV*, 80 consecutive out-ward patients at the Rheumatology Department, Karolinska University Hospital Huddinge were included, table 3. The patients should be aged 18– 80 years, have a diagnosis of RA and have disease duration of ≥1 year. The exclusion criteria were the same as in *paper I*.

Table 3. Clinical characteristics of the 80 out-ward RA patients

	Women, N=61	**Men, N=19**
Age (years)	60.8 (57.3-64.4)	63.4 (59.8-66.9)
Disease duration (years) *	6 (2-15)	5 (3-9)
DAS28	3.3 (3.0-3.6)	2.6 (2.1-3.0)
HAQ score	0.7 (0.5-0.8)	0.5 (0.2-0.7)

*= median. Data is presented as mean (CI) for normally distributed variables, and as median (inter-quartile range) for non-parametric variables. N=number

Paper III, 66 patients with RA according to ACR criteria were enrolled in the original study [47] and were recruited from Karolinska University Hospital, Solna and Södersjukhuset in Stockholm. They were eligible for inclusion if they were between 20 and 69 year, had disease duration between 2 and 10 year, had not tried dietary manipulation earlier, did not have a history of food sensitivity or food allergy and had active disease. Active disease was present if the patients fulfilled two of the following three criteria: duration of early morning stiffness ≥1 hour; ESR ≥30 mm; and six or more swollen and/or tender joints. Furthermore, they should be on stable doses of non-steroidal anti-inflammatory drugs, oral glucocorticoides (≤7.5 mg prednisolone) and disease modifying anti rheumatic drugs (DMARDs). All patients additionally received 1 mg/day vitamin B12 and 50 µg/day selenium in order to prevent dietary deficiency.
The patients were randomized to receive either a vegan diet free of gluten (n=38) or a non-vegan diet (n=28) for one year. Thirty patients in the vegan group and 28 in the non-vegan group completed at least 3 months or more on the diet regimen and where included in the present analyses.

3.2 METHODS

3.2.1 Clinical and body composition assessments

In all patients, disease activity was assessed by disease activity score including 28 joints (DAS28) [93] and functional status was estimated using the Swedish version of the Stanford Health Assessment Questionnaire (HAQ), a self-reported instrument measuring capacity to perform activities of daily living [33] *(paper I-IV).*

Supine blood pressure was recorded after 30 minutes of rest. Systolic and diastolic blood pressures were read to the nearest 5 mm Hg and the mean of two values was used in the analyses. Hypertension was defined as a blood pressure above 140/90 mm Hg or treatment with anti-hypertensive drugs [68] (*paper IV).*

All patients were weighed to the nearest 0.1 kg. Their standing height was measured to the nearest 0.1 cm. After that BMI was calculated from weight/height² (kg/m²). According to WHO standards, individuals with BMI values <18.5 kg/m² are considered underweight/malnourished, between 18.5 and 24.9 kg/m^2 as normal, 25–29.9 kg/m² as overweight and values greater than 30 kg/m² indicate obesity [131] *(paper I-IV).*

Triceps skin fold thickness (TSF), which reflects FM, was measured over the triceps at the midpoint between the acromion and olecranon using a Harpenden Caliper. The measurements were made in triplicate to the nearest 0.1 mm and the results averaged. Mid-arm circumference (MAC) was measured at the same level as TSF. Arm muscle circumference (AMC), an indicator of muscle protein stores, was calculated using the formula AMC (cm) =MAC (cm)–3.14xTSF (cm). The same two observers using the same equipment made all anthropometrical measurements. For TSF and AMC,

reference values from a Swedish population were used, matched for age and sex [115]. These measurements were performed on patients in *paper I*.

To identify central obesity waist circumference (WC) was measured on patients in *paper II* and *IV*, using a plastic inelastic flexible belt-type measuring tape. The patients were standing and the waist was measured to the nearest 0.5 cm midway between the iliac crest and the lower rib margin. According to the International Diabetes Federation (IDF) a waist circumference less than 94 cm in men and 80 cm in women would be low risk; an intermediate risk is indicated by 94–101.9 cm in men and 80–87.9 cm in women. Values above 102 cm in men and 88 cm in women indicate an increased risk for developing type 2 diabetes, coronary heart disease or hypertension [6].

The metabolic syndrome (MetS) was defined in *paper II* and *IV*, according to the IDF, i.e. presence of central obesity (waist perimeter ≥80 cm in females and ≥94 cm in males) plus two of the following four criteria; hypertriglyceridaemia (triglycerides ≥1.7mmol/1 or specific treatment), unfavourable HDL level (<1.3 mmol/1 in females and <1.1 mmol/1 in males and or specific lipid lowering treatment), hypertension (systolic BP ≥130 mm Hg or diastolic BP ≥85 mm Hg or specific antihypertensive treatment) and fasting plasma glucose ≥5.6 mmol/1 or prior diagnosis of type 2 diabetes mellitus [5].

Body composition was determined by whole-body DXA (the GE-Lunar Prodigy, software enCore 2006, version 10, 20,105, Madison, USA), in *paper I, II* and *IV*. Briefly, using specific anatomic landmarks, legs, arms, and trunk are isolated on the skeletal X-ray anterior view planogram using the DXA system's automated software. The DXA software then provides compositional estimates of legs, arms, trunk, head, and whole-body. The instrument automatically alters scan depth depending on thickness of the subject, as estimated from age, height and weight.

Body composition was also measured by BIA with the Tanita BC-418 8-contact electrode system (Tanita Corp., Tokyo, Japan) in *paper II.* There are four separate foot-pad electrodes mounted on the system's base and two electrodes in each of the hand grips. The eight electrodes are connected to a digital circuit board that electronically switches the electrical circuit under study. A predefined signal is passed through injector electrodes and impedance across the subject's tissues is measured with receiver electrodes. All measurements are carried out at 50 kHz with a 0.8 mA sine wave constant current.

Both DXA and BIA assessments were performed with the patients wearing light indoor clothing and with no detachable metal objects present.

The DXA and BIA expressed FFM and FM in absolute (kg) as well as in relative terms (as percent of total mass). FFM is the sum of lean body mass (LBM) and bone mineral. Since absolute FFM and FM are dependent of height, the fat free mass index (FFMI; kg/m^2) and fat mass index (FMI; kg/m^2) were calculated. An age and sex matched European reference population, i.e. 2982 men and 2647 women from Switzerland, allowed us to classify our patients according to body composition categories [103]. Cut-off values for low muscle mass and malnutrition were defined as FFMI values below the 10th percentile, corresponding to FFMI below 13.7-14.7 kg/m^2 for women and 16.9-17.6 kg/m^2 for men, depending on age. Obesity was defined as FMI above the 90th percentile, corresponding to FMI above 8.8-13.5 kg/m^2 for women and 7.2-9.0 kg/m^2 for men, also depending on age [103]. The desirable levels for FM% are 20 to 30% for women and 12 to 20% for men [1].

3.2.1 Nutritional and dietary assessments

Nutritional status was assessed by the use of the following questionnaires: Mini Nutritional Assessment (MNA) [44, 128] *(paper I* and *II)* and the Subjective Global Assessment (SGA, Swedish version) [25, 91] *(paper I).* Two screening tools were also used, the Malnutrition Universal Screening Tool (MUST), developed by British Association for Parenteral and Enteral Nutrition (BAPEN) [112, 127] and the Nutritional Risk Screening tool 2002 (NRS-2002), endorsed by the European Society for Clinical Nutrition (ESPEN) [61, 62] *(paper I).*

In *paper III,* dietary intake records during three days assessed the compliance with the dietary treatment every third month. The gluten-free vegan diet contained vegetables, root vegetables, nuts and fruits as well as buckwheat, millet, corn, rice and sunflower seeds. The non-vegan diet consisted of a variety of foods from all food groups.

In *paper IV* the patients were asked to report their frequency of use of 88 food items over the past year, using FFQ. The nutrient calculations were carried out using nutrient composition values from the Swedish National Food Administration data [13]. The mediterranean diet was defined, as described in FFQ, as a diet with high intake of fruits, vegetables, legumes, nuts, olive oil, fish, shellfish, lean meat and a minimum of dairy products [74] *(paper IV).*

3.2.1 Biochemical measures and abdominal fat cell analyses

Venous blood samples were drawn between 07:30 and 10:00 after an overnight fast. In *paper III* data and serum samples were obtained at time for diet randomization and after 3 and 12 months. The biochemical variables were determined by standard laboratory methods with commercial kits. They included C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), plasma glucose, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides and rheumatoid factor (RF). The serum lipid concentrations were considered pathologic when total cholesterol was >5.0 mmol/L, LDL ≥3.0 mmol/L, triglycerides ≥1.7 mmol/L, for women HDL <1.3 mmol/L and for men HDL <1.1 mmol/L *(paper I-IV)*.

Samples, which had been stored at –70 C, were analyzed for oxLDL, anti-PC and abdominal fat. OxLDL and anti-PC were determined by use of a commercial kits, Mercodia, Uppsala, Sweden over the past year and Athera CVDefine™, Stockholm, Sweden, respectively as described by the manufacturers *(paper III* and *IV).*

Subcutaneous abdominal fat cells were aspirated with a needle attached to a vacuum tube and analysed for fatty acids. The fatty acid composition was analysed by gas liquid chromatography [16]. The amounts of fatty acids were given as the relative percentage of the sum of the FAs analysed *(paper IV).*

3.3 ETHICAL ASPECTS

The studies were approved by the Regional Ethics Committees of Karolinska University Hospital Huddinge, of Södersjukhuset, or of Karolinska Institutet Stockholm, Sweden. For the studies presented in *papers I-IV* all patients gave their informed consent before inclusion.

3.4 STATISTICS

The statistical analysis program STATISTICA 7, Stat Soft Scandinavia AB, was used for statistical analysis.

In all papers p-values <0.05 were considered significant. Data were presented as mean (confidence interval) or median (interquartile range) depending if the data were normally distributed or not *(paper I-IV).* Differences between groups were assessed using Student *t* test and Mann-Whitney U test depending on the distribution of the analyzed variable *(paper I-IV*). Anova was also used in *paper II* and *III* to compare differences among groups. In *paper II, III* and *IV* correlation analysis was performed using simple regression for normally distributed variables, and Spearman´s correlation analysis for non-normally distributed variables. Multiple linear regression analyses were used in *paper I* and *II.* Skewed continuous variables were logarithmically transformed to attain a normal distribution. In *paper I* sensitivity and specificity were calculated to evaluate the different assessment tools/methods with FFMI as "gold standard". Sensitivity = true positive / (true positive + false negative) x 100 i.e. the proportion of ill (here malnourished) patients identified by the test. Specificity = true negative / (true negative + false positive) x 100 i.e. the proportion of healthy (here well nourished) patients identified by the test. In *paper II* the method of Bland and Altman [15] was used to assess agreement between BIA and DXA. The mean difference between the methods (bias) and the ± 2 SDs of the difference between methods (limits of agreement) were calculated. Ninety-five percent of the data points should be within the ±2 SDs of the mean difference.

4 RESULTS AND DISCUSSION

4.1 BODY COMPOSITION AND NUTRITIONAL STATUS

A large proportion of both the in- and out-ward RA patients had BMI in the overweight/obese range (BMI >25) (*paper I, II)*. Further 57% of the women (WC >80 cm) and 89% of the men (WC >94 cm) were centrally obese (*paper II, IV*). Twenty percent of the women and 63% of the men could be diagnosed to have MetS (*paper IV*).

According to FMI, analysed with DXA, 42% of the women and 53% of the men, admitted to our in-ward, could be classified as obese. Corresponding figures for the out-ward women and men were 31% and 53%, respectively. Despite that, FFMI was low in 52% and 30% of the in-ward women and men, respectively. Corresponding figures for the out-ward women and men were 26% and 21%, respectively *(paper I, II).* It thus became evident that the concurrent elevation of FM and decreased FFM made BMI a non-reliable tool to detect malnutrition (*paper I, II).*

Multiple regression analyses revealed gender ($p< 0.001$), ESR ($p=0.011$), duration of RA ($p= 0.027$), age ($p=0.022$) and HAQ trendwise ($p=0.058$) to be independently related with FFM. FM% correlated significantly with DAS28 ($r = 0.24$, $p=0.03$) and ESR ($r = 0.45$, $p<0.001$) *(paper II).*

Percentiles for FFMI and FMI from a large Swiss population consisting of healthy adults were used to define malnutrition (*paper I, II*). Thus a FFMI below the 10th percentile was used as cut-off for low FFM, a value corresponding to BMI <18.5. We found it appropriate to use this cut-off since we had decided to use WHO's classification for underweight/malnutrition, i.e. BMI <18.5 [131]. However, in clinical practice and especially in older subjects also BMI of at least 20.0 has been considered appropriate [23] as cut off for malnutrition. The cut-off used for obesity, FMI above the 90th percentile, corresponds to a BMI >30.

We felt it appropriate to use the external reference population *(paper I, II)* from Switzerland as this population is similar to the Swedish with respect to ethnicity and as body composition is not only dependent on age and gender but also varies with ethnicity [26]. This Swiss population also had the advantage of being close in time with our study. Taking into account that people in most of the western world are getting more obese in time, it is important to use a reference population from the same decade.

The high frequency of reduced FFM, in the out-ward patients *(paper II)*, was somewhat surprisingly as the RA disease was fairly well controlled concerning inflammatory activity and physical function and as rheumatoid cachexia has been postulated to be associated with excess production of inflammatory cytokines and low physical activity [29, 94]. A possible explanation is that the derangement of body composition is a result of several years of disease with varying inflammatory activity in combination with the

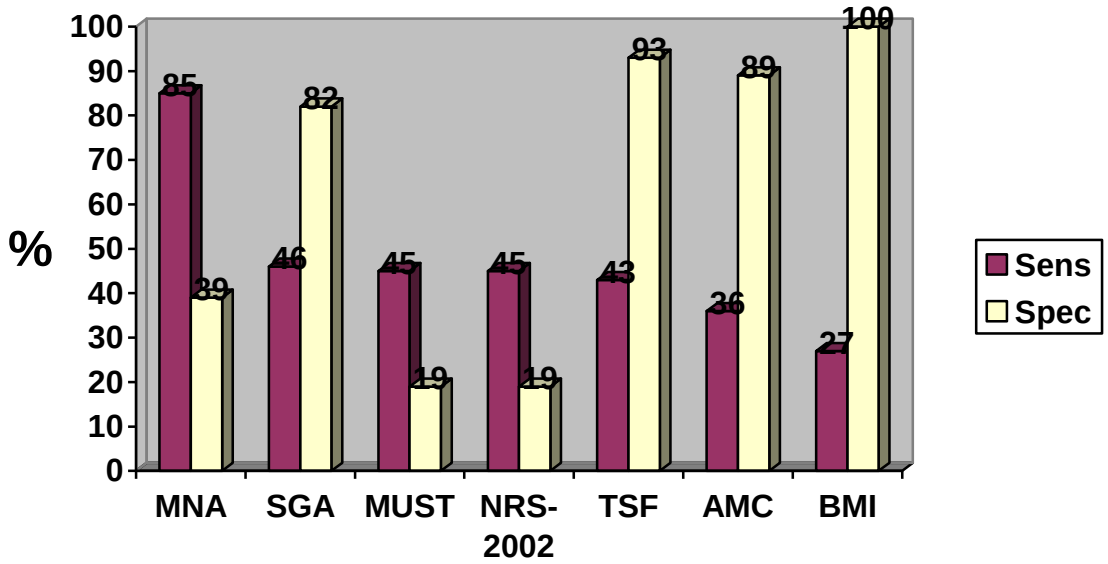
actual low-grade inflammation, corresponding to the low-grade acute reaction associated with protein-energy malnutrition in chronically ill patients [20]. This possibility was confirmed by the found negative correlation between FFM and disease duration as well as age and also between FFM and disease activity (DAS28) and functional status (HAQ).

FFM is mainly composed of skeletal muscle and it was therefore not unexpected to find the negative correlation between FFM and HAQ score, even if a high HAQ may not depend only on muscle strength but also on joint destruction. As stated above we suggest that the reduction of FFM depended partly on pro-inflammatory cytokines, but a further cause could be physical inactivity [122]. This possibility could not be proven in our studies as we only had information about disability and not about physical activity. However, it is shown that high intensity progressive resistance training may significantly increase FFM, especially in skeletal muscles [71].

Interestingly we found that FM% correlated significantly, and positively, with inflammatory markers as ESR and DAS28 *(paper II)*. Adipose tissue is known to produce cytokines like TNF- α and IL-6 [49] and adiposity is independently associated with CRP levels in women with RA [40]. This association may confound the estimation of RA disease activity when serum CRP concentration is used as a surrogate for systemic inflammation. CRP is not directly expressed by adipose tissue, but its synthesis in the liver is induced by a number of adipose-derived cytokines, principally IL-6 [40].

The anthropometrical measurements BMI, AMC and TSF showed low sensitivity and high specificity for detecting malnutrition, i.e. many malnourished should have been missed using these assessments tools, figure 5. AMC in both RA women and men and TSF in women were similar to a Swedish normal population. Only in RA men, TSF were significantly higher than the male reference population ($p<0.001$) [115] *(paper I).*

Figure 5. Sensitivity and specificity for the anthropometrical assessments and the nutritional questionnaires to detect malnutrition as determined by FFMI.



Among the nutritional evaluation tools, MNA had a poor specificity but a fairly good sensitivity to detect malnutrition, figure 5. Consequently several of our patients were misclassified by MNA. The questions in MNA focus on changes in weight, changes in food intake, mobility, psychological stress and acute disease in the past 3 months. The rest of the questions focus to a higher extent on the present period, such as type of dwelling, quality of dietary intake, presence of sores, ability to eat and transfer and how the patients consider themselves according to nutritional problems and health status. Our results suggest that MNA assesses poor health, among RA patients, rather than nutritional status. In support of this argumentation is the low specificity and the negative correlations between MNA and RA disease duration, DAS28, ESR, and HAQ *(paper I, II).* In *paper I* we suggested MNA to be used as a screening instrument, but because of its low specificity it should be followed by body composition assessment.

SGA, in contrast to MNA, had a fairly good specificity but a poor sensitivity to detect malnutrition. The reason for the low sensitivity probably depended on the aberrations in body composition. Our patients had gained fat, not lost it. Further the low sensitivity may be due to that the questions asked are focusing on events weeks back in time (except for weight). The changes in our patients had occurred over a longer time period, perhaps one year or more and their weight had not changed much over time. The concurrent gain of fat and loss of FFM made it difficult to detect malnutrition when the patient was physically examined. There were only few much-deteriorated patients (in our population), who had even lost their fat and these patients were identified by SGA (SGA C) *(paper I).*

MUST and NRS-2002 screened the majority of our patients as low risk for malnutrition. The reasons for this misjudgement are probably that both instruments focus on BMI, weight loss and acute illness. These variables appear to be poor determinants of malnutrition in patients with RA, which mainly depends on cachexia and not on wasting. NRS-2002 focuses on the same issues as MUST, issues such as reduced dietary intake during the last week and impaired general condition. The NRS-2002 also takes in consideration old age and severity of disease *(paper I).*

4.1.1 Rheumatoid cachexia

As discussed in this thesis rheumatoid cachexia is defined as loss of muscle mass in presence of stable or increased fat mass. Patients with RA often have stable weight and normal or high BMI, which makes this form of cachexia often underestimated and frequently overlooked. Mostly the increased morbidity associated with rheumatoid cachexia has been attributing the loss of body lean mass whereas the coincidental increase of fat mass has not been considered in this context. We therefore tried to find cut-off levels for FFM and FM associated with increased morbidity in this condition.

By using the definition of FFMI below the 10th percentile in combination with FMI above the 25th percentile, 38% of the in-ward women and 30% of the men were categorized to have rheumatoid cachexia (*paper I).* Corresponding figures for the out-ward patients were 18% and 21%, respectively, *(paper IV).* When using the definition of FFMI below the 25th percentile and FMI above the 50th percentile 18% of the out-ward women and 26% of the men were assessed to have rheumatoid cachexia *(paper IV).* In that paper we were able to show that patients with rheumatoid cachexia according to the last mentioned definition had higher serum LDL (p=0.029), cholesterol (p=0.033) and trendwise oxLDL (0.056) as well as significantly lower levels of anti-PC IgM (p=0.040), than those without this derangement in their body composition.

Furthermore, a large proportion of the patients (69%) with rheumatoid cachexia (FFMI below the 25th percentile and FMI above the 50th percentile) had hypertension a condition associated with high levels of oxLDL and low of anti-PC. One novel finding was that anti-PC levels were decreased in patients with rheumatoid cachexia. Low anti-PC could predispose to cardiovascular disease [105, 113] and might thus be one explanation for the increased morbidity in rheumatoid cachexia.

Due to these findings we proposed FFMI below the 25th percentile in combination with FMI above the 50th percentile as the definition for rheumatoid cachexia.

4.2 COMPARISON OF BIA AND DXA (PAPER II)

With DXA as "gold standard" we wanted to investigate if BIA could be used in clinical practice to assess body composition. Our results reviled that there was a significant variation in body composition outcomes between DXA and BIA. Even though there was a relative good agreement between the methods, BIA underestimated FM (by 4.3% and 2.2 kg) and overestimated FFM in absolute terms. Consistent with other reports [82, 90, 114] we could demonstrate wide limits of agreement between the two methods. Thus, although high correlation coefficients indicate good relative agreement, correlation analysis alone is not sufficient to verify the degree of coincidence among the methods [15].

We found a systematic bias causing BIA to overestimate FFM by 2 kg compared with DXA. When using BIA, one option would be to correct the FFM value obtained with BIA with 2 kg. However, we still have to accept the wide limits of agreement by approximately ±5 kg. Although the situation is not ideal, this action could be a way to assess the RA patient's body composition in clinical practice.

BIA might prove particularly useful for follow-up evaluations after for example pharmacological treatments, physical activity or weight loss interventions. Thus the BIA method was found to be a useful, valid method to assess changes in body composition in young obese women during weight loss [117]. However, increasing discrepancies between DXA and BIA have been observed when studying larger fat losses, implying that the BIA equipment may have limited validity in this situation [83].

4.3 DIET AND THE RA DISEASE

In *paper III* we were able to show that the RA patients on a gluten-free vegan diet achieved significantly reduced DAS28 and HAQ score after both 3 and 12 months and CRP after 12 months. Among the non-vegan diet group, DAS28 had decrease at 3 but not 12 months, whereas HAQ score and CRP were unchanged over time. In the vegan group 63% were good and moderate responders at 12 months, according to the EULAR response criteria [125], compared with 32% of the non-vegan diet patients.

In *paper IV* the majority of the 80 patients reported to follow a normal western diet, whereas 15 women followed a vegetarian or mediterranean like diet. In average they reported a lower energy intake than recommended. However, the low energy intake was mainly reported by patients with moderate inflammatory activity. Thus, female patients with DAS28 >3.2 had a lower mean energy intake than those with DAS28 ≤3.2, 1500 kcal/d vs. 1800 kcal/d, p=0.016.

If the low energy intake is real or a consequence of underreporting is uncertain. However, underreporting seems most probable as earlier comparison between FFQ and weighed diet records concerning fat intake showed lower total intake registered by FFQ than the weighed diet, whereas the proportions of fat components were similar [137]. Though, research suggests that underreporting of fat intake exists but is greater among individuals that are overweight [87].

The patients fat intake consisted of more saturated and less unsaturated FAs than recommended [3]. However, patients with or without cachexia did not differ with respect to dietary fat intake or intake of mediterranean like diet.

Intake of SFAs is suggested to be associated with the development of MetS and CVD [126] and higher habitual intakes of SFAs are independently associated with increased atherosclerosis [73]. These consequences support current dietary guidelines [3] and would urge the clinician to recommend the RA patient to replace SFAs with PUFAs in the prevention of metabolic and cardiovascular diseases and a possibility also to reduce inflammation.

4.3.1 Fatty acids in adipose tissue, *paper IV*

The reliability of the long-term FA intake according to the FFQ was verified by the significant correlations found between the PUFAs ingested and those in adipose tissue. Thus the reported dietary intake of FA correlated significantly with that in adipose tissue as to FA 14:0 (myristic acid), 18:2 n-6 (linoleic acid), 18:3 n-3 (alfa-linolenic acid), 20:4 n-6 (arachidonic acid), 20:5 n-3 (EPA), 22:5 n-3 (DPA) and 22:6 n-3 (DHA), but not with 16:0 (palmitic acid) and 18:1 n-9 (oleic acid).

PUFAs in adipose tissue are largely exogenous and hence valid markers to assess dietary intake [12, 136]. In contrast, SFAs and MUFA are not considered to be good biomarkers of intake because they are also endogenously synthesized [12, 53], which was evident in the RA patients, with weaker correlations concerning 16:0 and 18:1 n-9. Furthermore, the noted significant correlation between 14:0 in diet and adipose tissue verifies a report by Wolk et al that also this fatty acid is a valid biomarker for fatty acid intake [135].

When analysing the adipose FA content for the different self-reported diet groups it was found that subjects compliant to mediterranean like or vegetarian diets had a more favourable FA profile, i.e. with higher PUFAs than those reporting to eat a normal western diet.

4.3.1 Serum Lipids and anti-PC in RA

As diet is of importance for the lipoprotein profile it was of interest to study if a vegan diet, with its low content of saturated fat and higher content of polyunsaturated fat, could affect serum lipids and the natural atheroprotective anti-PC in patients with RA, *paper III.*

In the patients randomized to gluten-free vegan diet, LDL and cholesterol decreased after both 3 and 12 months ($p<0.01$) and oxLDL after 3 months ($p=0.021$) and trendwise after 12 months ($p=0.090$). IgM anti-PC increased trendwise after 12 months ($p=0.057$). In the control diet group, IgM anti-PC decreased both after 3 and 12 months ($p<0.01$). When we separated vegan patients into clinical responders and non-responders at 12 months (according to DAS28 response), the effects on oxLDL was seen only in responders ($p<0.05$).

This study indicates that a gluten-free vegan diet in RA induces changes that are potentially atheroprotective and anti-inflammatory, including decreased LDL and oxLDL levels and raised anti-PC IgM. It is possible that the reduction in oxLDL also could contribute to the decreased disease activity, a possibility supported by the finding that the reduction of oxLDL was seen only in diet responders.

Cross-sectional, 53% of the women with RA and 32% of the men had cholesterol >5.0 mmol/l and low HDL levels were found in 3% of the women and 5% in the men (*paper IV*). High LDL levels were present in 51% of the women and in 32% of the men. For the RA women these lipid values did not differ between those with normal diet and those with the mediterranean like/vegetarian diets. However, anti-PC IgM levels differed significantly between patients in the different self-reported diet groups. Thus, the women with mediterranean like diet had significantly higher median levels, compared with those with self-reported normal western diet, 198.2 (171.6-209.2) vs. 43.5 (30.3-65.5), $p<0.001$.

The very high levels of anti-PC in patients on mediterranean diet is of great interest as subjects on this diet are reported to have lower frequency of CVD [88, 132]. Also the low levels of anti-PC levels in the RA patients with cachexia, described above (see the heading rheumatoid cachexia), is of interest as low anti-PC could predispose to CVD [105, 113] and might thus be one explanation for the increased morbidity in rheumatoid cachexia. The cause of this association is not known. In RA, however, anti-PC has not been studied in relation to CVD.

As described above patients with rheumatoid cachexia also had dyslipidemia (*paper IV*). Of the lipids oxLDL might be especially important as it has proinflammatory and immune stimulatory properties [34, 35]. OxLDL has been detected in synovia from RA patients [133] and is suggested to be of importance in the pathogenesis of CVD [14]. Even if there are no existing reference values for oxLDL the present patients had higher levels than earlier reported for healthy people, 62.1 (55.7-68.4) vs. 42.2 U/l (±14.7) and

48 U/l (35-68) [57, 86]. Also compared with RA patients from Korea [57] the present patients had higher levels, a difference that might be secondary to different diet habits.

5 GENERAL SUMMARY

When I started this thesis the aim was to explore the nutritional status in patients with RA. That because the literature reviled large discrepancy between studies concerning prevalence of malnutrition in these patients, ranging from 26% to 71%. However, there were no validated nutritional assessment tools for RA patients. The first step to dissolve this shortage was to validate different nutritional assessment tools. To be able to assess nutritional status you also have to consider body composition and for that purpose DXA measurement was chosen as a "gold standard". In this process we ran into difficulties because there were no agreements on the definitions either on malnutrition or rheumatoid cachexia.

We decided to use the WHO's definition of malnutrition, which is "the cellular imbalance between supply of nutrients and energy and the body's demand for them to ensure growth, maintenance and specific functions" [130]. This is a broad definition and is used worldwide. Concerning rheumatoid cachexia we had to set a definition especially for RA patients, since there is opinions in the nutritional world that cachexia is defined as loss of both FFM and FM. In our opinion we need the definition rheumatoid cachexia, which is loss of FFM and gain of FM. Using this definition we can separate RA patients from for example patients with cancer cachexia.

In *paper I* 60 in-ward patients were recruited, patients with high disease activity and therefore supposed to be malnourished. They were assessed by anthropometric measures and the nutritional assessment and screening tools MNA, SGA, MUST and NRS-2002. Body composition was determined by DXA. Twelve per cent of the women and none of the few men had BMI <18.5, i.e. the cut-off value for malnutrition, whereas FFMI indicated 52% of the women and 30% of the men to be malnourished. Concurrent elevation of FM made BMI a non-reliable tool to detect the malnutrition. Among the nutritional tools MNA had the highest sensitivity to identify malnourished RA patients. Therefore, MNA was proposed to be used as a screening instrument, followed by determination of body composition.

In *paper II* nutritional status and body composition was further evaluated in out-ward patients, also with the aim to explore the feasibility of BIA in clinical practice. These patients were in general centrally obese and about every fifth patient displayed concomitant low FFMI and elevated FMI, i.e. rheumatoid cachexia. BMI and MNA were not able to detect this condition in these patients. Reduced FFM was independently related to age, disease duration and ESR. Compared with DXA, BIA underestimated FM and overestimated FFM. There was a systematic bias, where BIA overestimated FFM by 2 kg. When using BIA, one option would be to correct the BIA FFM value by 2 kg.

As discussed earlier, and shown in paper I and II, reduced FFM and elevated FM were common in patients with RA. However, there is no consensus as to cut-off values for rheumatoid cachexia, which is troublesome as this condition is associated with increased morbidity. In *paper IV* we evaluated 2 possible definitions, of which FFMI below the 25th percentile together with FMI above the 50th percentile of the reference population was associated with dyslipidemia such as high levels of oxLDL and low levels of the atheroprotective anti-PC as well as increased frequency of hypertension. We therefore proposed this definition to be used.

Further we analyzed the importance of diet for rheumatoid cachexia and dyslipidemia. The out-ward RA patients in *paper IV* reported a low intake of total energy, according to FFQ and a high intake of SFAs. This high intake was also reflected in the fatty acid composition of the adipose tissue. However we could not find that the fatty acid intake differed between cachectic and not cachectic patients. Adherences to a mediterranean like diet did not either differ between these conditions. The patients on mediterranean like or vegetarian diets had though a more favourable FA profile in adipose tissue, i.e. higher PUFAs than those reporting compliance to a normal western diet.

Patients on mediterranean like diet had significantly higher levels of anti-PC in serum compared with those reporting a normal western diet (*paper IV)*. This is in line with the findings in the randomized study comparing gluten free vegan diet with normal western diet during one year (*paper III*). The patients on vegan diet improved as to clinical status and inflammatory markers. Further these patients got lower LDL, oxLDL and higher anti-PC IgM compared with those on normal western diet, thus changes that are potentially anti-inflammatory and atheroprotective.

6 SVENSK SAMMANFATTNING

Reumatoid artrit (RA) är en inflammatorisk systemsjukdom som drabbar ca 0,5 procent av Sveriges befolkning. De flesta insjuknar när de är i 45-65 årsåldern. RA sjukdomen förekommer oftare hos kvinnor än hos män och risken att få sjukdomen är större för rökare än icke rökare, särskilt hos den som har specifika riskgener. Patienter med RA har en ökad dödlighet i kardiovaskulär sjukdom.

Sjukdomen kännetecknas av inflammation i kroppens leder, framförallt i händer och fötter. Den inflammerade ledhinnan har förmåga att bryta ned brosk och närliggande ben. Förutom symtom från leder och skelett leder den kroniska inflammationen till ett katabolt tillstånd. Detta medför ökad risk för malnutrition med förlorad muskelmassa och samtidigt ofta ökad fettmassa, ett fenomen kallat reumatoid kakexi. Förutom av den inflammatoriska processen, kan patienter med RA bli malnutrierad också beroende på dålig aptit, förändring i kost och fysiska funktionshinder. Många RA patienter lider av undernäring utan att detta uppmärksammas i vården. För att kunna identifiera vilka patienter, som är eller löper risk att bli malnutrierade, syftade denna avhandling till att ta reda på vilka instrument/metoder som tillförlitligast bedömer näringstillståndet. Dessutom var målen att studera förekomsten av reumatoid kakexi, kostens betydelse för detta tillstånd samt inverkan av kost och reumatoid kakexi på dyslipidemi och andra riskfaktorer för hjärt-kärlsjukdom.

I *delstudie I* fann vi att 89% av 60 RA-patienter i slutenvård med förhöjd inflammatorisk aktivitet och nedsatt funktion hade normalt eller förhöjt BMI. Trots normalt BMI bedömdes en stor andel av patienterna som malnutrierade eller under risk att utveckla malnutrition med hjälp av nutritionsformulären MNA, SGA, MUST and NRS-2002. Mätt med dual x-ray absorptiometry (DXA) hade 52% av kvinnorna och 30% av männen låg fettfri massa, mätt som fettfri mass-index (FFMI), och bedömdes därigenom som malnutrierade. Många hade samtidigt ökad fettmassa. Med BMI upptäcktes inte denna malnutrition hos RA patienterna. Av nutritionsformulären hade MNA (Mini Nutritional Assessment) den största sensitiviteten att identifiera de flesta. MNA föreslogs därför kunna användas som screening instrument för att identifiera malnutrition vid RA, eventuellt följt av DXA.

I *delstudie II* studerades kroppssammansättning och malnutrition hos RA patienter i öppenvård, samt även om kroppssammansättningen kunde mätas med bioelektrisk impedans (BIA) med samma noggrannhet som DXA. Studien visade att minskad muskelmassa, mätt med DXA, och central fetma var vanligt även hos dessa patienter, med låg inflammatorisk aktivitet och god funktion. Tjugosex procent av kvinnorna och 21% av männen bedömdes som malnutrierade och en av fem hade reumatoid kakexi, förekomst av samtidig låg FFMI och ökad fett mass-index (FMI). Varken med BMI eller med MNA kunde detta tillstånd upptäckas. Reducerad FFM var oberoende relaterad till ålder, sjukdomsduration och sänka. Vid jämförelse mellan BIA och DXA,

som brukar användas som ”gold standard”, fann vi att dessa metoder stämde ganska väl överens men att BIA undervärderade fettmassa och övervärderade fett fria massan, den senare med ungefär 2 kg.

I *delstudie III* har vi i en jämförande kontrollerad studie med totalt 66 patienter visat att glutenfri vegankost under 1 år medförde minskad inflammatorisk aktivitet, vikt och BMI. Vi undersökte också om vegankosten innebar förändring av lipider såsom oxLDL (oxiderat LDL) och antiphosphorylcolin IgM (anti-PC IgM). Vi fann minskade serum nivåer av LDL och oxLDL men ökad halt av anti-PC. Vegankosten inducerade således förändringar som är potentiellt både ateroprotektiva och antiinflammatoriska. Detta är intressant inte minst pga. det påvisade sambandet mellan RA och ateroskleros.

I *delstudie IV* studerades kostens betydelse för kroppssammasättningen och dyslipidemin. RA pateinter i öppen vård rapporterade ett lågt totalt energiintag och ett högt intag av mättade fetter, som avspeglade sig i fettsyrainnehållet i bukfett. Det var främst patienter med medelhög till hög sjukdomsaktivitet som rapporterade ett lägre energiintag. Fettsyraintaget skiljde sig inte åt mellan patienten med eller utan kakexi. De patienter som rapporterade att de åt en medelhavslik eller vegetarisk kost hade högre nivåer av fleromättade fetter i sin fettväv än de som rapporterade att de åt en ”vanlig” kost. Vi fann också att de patienter som åt medelhavslik kost och vegankost hade lägre nivåer av blodfetterna LDL och oxLDL samt högre nivåer av anti-PC IgM.

Vidare utvärderades två olika definitioner på reumatoid kakexi, med syftet att finna om någon av dessa medförde en ökad sjuklighet. Vi fann att vid ett tillstånd med fettfri massa motsvarande BMI <20 (FFMI under 25^{e} percentilen jämfört med en normalpopulation) tillsammans med fett massa motsvarande BMI >25 (FMI över 50^{e} percentilen) ökade andelen patienter med hypertoni. Dessa patinter hade även höga blodfetter, framför allt LDL och oxLDL samt låga nivåer av anti-PC IgM. Därför föreslog vi användande av denna definition för reumatoid kakexi.

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