FACULTY OF MEDICINE AND HEALTH SCIENCES

THE IMPACT OF OBESITY ON RHEUMATOID ARTHRITIS PROGRESSION: MOLECULAR MECHANISMS

Jeroen Preem
Student number: 01409327

Supervisor: Prof. Dr. Karolien De Bosscher

A dissertation submitted to Ghent University in partial fulfilment of the requirements for the degree of Master of Medicine in Medicine

Academic year: 2020 – 2021

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Preface

This dissertation is the result of a process that started in April of 2019. Now, 20 months later, I can present you this essay. When the subject of my dissertation was officially communicated, I had not received any lecture about rheumatoid arthritis. Obesity was a concept of which I had some notions, but the pathophysiology behind it was unclear. To involve molecular mechanisms and mice models, topics that are not emphasized during the education of a medicine student, was challenging for me. However, these challenges have motivated me to acquire new knowledge, of which I am convinced some will help me during my career, whatever way it will go. During the writing process, I have learned to appreciate the complexity of the pathophysiology behind obesity and rheumatoid arthritis and to not underestimate the impact obesity may have on a wide range of pathologies. Due to the rising worldwide prevalence of obesity, I am convinced of the added value of my work to others and myself. In this preface, I would like to thank some people who have helped me during this experience of writing my dissertation.

First of all, I would like to thank my supervisor, Prof. Dr. Karolien De Bosscher. Starting from the first meeting, she made me feel comfortable about the writing process and was clear in what she expected from me. Due to her long expertise in the field of biomolecular medicine, I was supported in the best possible way. She put enormous effort in guiding me, in correcting my sometimes sloppy English and in helping me to reflect critically on my own text. Thanks as well to Dr. Sofie Desmet who helped with some final suggestions.

I would like to thank some people who helped me during the writing. The writing process was a long-term operation with its ups and downs and I could not have done it without my friends and family who supported me. Thank you to Laura and Annabel, my study buddies on which I could always count and who I spent so many hours with working on our dissertations. Thank you to Emma and Freek, my online study buddies with who I could vent my emotions and who motivated me to work until the late hours. Special thanks to Thomas and Sébastien who read my thesis to check on spelling mistakes. Finally, I would like to thank my mom who provided me with the best circumstances to focus me on my thesis. Together with my father, she gave me the chance to study and chase my dreams. I hope my father would be proud of me.

With this dissertation, I finish the theoretical education of my medicine studies. I have put enormous effort in the following pages and I am proud of the result. I hope that you, as reader, will enjoy the interesting results as much as I did.

Jeroen Preem

Blankenberge, December 2020

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Abstract

In English

Objective: To summarize the impact of obesity on the progression of rheumatoid arthritis (RA), with special focus on the underlying molecular mechanisms explaining this association.

Methods: Different databases (PubMed, Embase, Web of Science and Google Scholar) were searched for studies on this topic by using relevant keywords. Selected studies have been analyzed extensively to first situate the background and next to summarize relevant results combined with own perspective and critical views.

Results: Most studies found worsened Disease Activity Score using 28 joint counts (DAS28) scores in obese RA-patients compared to non-obese RA-patients, resulting in fewer patients reaching disease remission in the obese population. Body Mass Index (BMI) negatively impacts Health Assessment Questionnaire (HAQ) and patient global assessment evaluated by the Visual Analogue Scale (VAS). However, elevated BMI is associated with less progression of radiographic damage, confirmed by studies using Magnetic Resonance Imaging (MRI). Both obesity and RA are linked with immune system alterations and characterized by a pro-inflammatory environment. Animal studies using collagen-induced arthritis (CIA) mice mainly found increased inflammatory markers in obese CIA-mice compared to normal weight CIA-mice. Obesity seems to aggravate the disease state of RA-patients, with effects of obesity being more pronounced in the early stages of RA and in remission. The pro-inflammatory effects of adipokines on the immune system have been suggested to explain these associations. Adiponectin is the only adipokine to have decreased levels in obese patients, possibly explaining the protective effects of obesity on bone damage. For leptin and other adipokines, inconsistent findings are observed. Leptin is linked with both pro-inflammatory effects and anti-inflammatory markers, possibly due to the phenomenon of leptin resistance. Other adipokines (resistin, chemerin, visfatin) were proven to play a role in the pathophysiology of obesity and RA, possibly predominantly by exerting pro-inflammatory effects, but the associations with disease parameters are controversial and the exact mechanisms are yet to be uncovered. It must be noted that for all aspects discussed, studies contradicting each other have been found.

Conclusion: Results suggest a negative impact of obesity on the global status of RA-patients with worsened disease activity, self-reported pain and disability. However, obesity may have protective effects on bone and cartilage damage in RA-patients. Obesity aggravates the inflammatory state in RA-patients by various effects on the immune system, mediated among others by adipokines. The impact of obesity on RA is more pronounced in early stages of RA and in remission. Further research is needed.

In het Nederlands

Doelstelling: De impact van obesitas op reumatoïde artritis (RA) onderzoeken, met bijzondere aandacht voor de achterliggende moleculaire mechanismen als verklaring.

Methodologie: Verschillende databanken (PubMed, Embase, Web of Science en Google Scholar) werden geraadpleegd om artikels te vinden, gebruik makende van relevante termen. De geselecteerde artikels werden uitgebreid geanalyseerd om de achtergrond te schetsen en de relevante resultaten samen te vatten, in combinatie met eigen inzichten en kritische bedenkingen.

Resultaten: De meeste studies stelden slechtere 'Disease Activity Score using 28 joint counts' (DAS28) scores, en zodus minder remissie, vast in obese RA-patiënten vergeleken met niet-obese RA-patiënten. Body Mass Index (BMI) heeft een negatieve impact op 'Health Assessment Questionnaire' (HAQ) en op de zelf-gerapporteerde globale beoordeling van de patiënt, geëvalueerd door een Visueel Analoge Schaal (VAS). Gestegen BMI is echter geassocieerd met minder progressie van radiografische schade, zoals bevestigd door 'Magnetic Resonance Imaging' (MRI). Zowel obesitas als RA zijn gelinkt aan veranderingen in het immuunsysteem en zijn gekarakteriseerd door een pro-inflammatoire omgeving. Diermodellen die gebruik maken van 'collagen-induced arthritis' (CIA) muizen hebben consequent verhoogde inflammatoire merkers vastgesteld in obese CIA-muizen vergeleken met CIA-muizen met een normaal gewicht. Obesitas lijkt de ziektestatus van RA-patiënten te verergeren, voornamelijk in de vroege stadia van RA en bij remissie. De pro-inflammatoire effecten van adipokines op het immuunsysteem zijn een mogelijke verklaring. Adiponectine is het enige adipokine dat door obesitas in concentratie verlaagd voorkomt, hetgeen mogelijks de beschermende effecten van obesitas op botschade kan verklaren. Voor leptine en andere adipokines werden tegenstrijdige resultaten gevonden. Leptine is gelinkt met zowel pro- als anti-inflammatoire effecten, mogelijks door het fenomeen van leptineresistentie. Ook andere adipokines (resistine, chemerine, visfatine) hebben een rol toebedeeld gekregen in de pathofysiologie van obesitas en RA, vermoedelijk eerder van pro-inflammatoire aard, echter, de associaties met de ziekteparameters zijn controversieel en de exacte onderliggende mechanismen dienen verder ontrafeld. Het is belangrijk mee te geven dat voor alle besproken bevindingen onderzoeksresultaten bestaan die elkaar tegenspreken.

Conclusie: De resultaten duiden op een negatieve impact van obesitas op de globale status van RA-patiënten met als gevolg een slechtere ziekteactiviteit, zelf-gerapporteerde pijn en invaliditeit. Obesitas toont echter beschermende effecten op bot- en kraakbeenschade bij RA-patiënten. Obesitas verergert de inflammatoire toestand bij RA-patiënten door, onder andere, adipokine-gemedieerde effecten op het immuunsysteem. De impact van obesitas op RA is meer uitgesproken in de vroege stadia van RA en in remissie. Verder onderzoek is aangeraden.

1. Introduction

1.1 Obesity

1.1.1 Definition and epidemiology

Obesity can be defined as the result of weight gain, originating from an imbalance between the storing of energy by the body and the consumption of it (1). Obesity is a condition associated with a wide spectrum of health disorders (2-4). An exorbitant accumulation of adipose tissue in the body characterizes the phenomenon of obesity (5). It has been a subject of interest since the period of the Ancient Greeks, with Hippocrates (456-375 BC) leading the way, but it has only been linked with comorbidities for less than a century (2). In fact, it was not until June 2013 for obesity to be labeled as a disease by 'The American Medical Association', which explains since then a vastly growing awareness worldwide of the enormous issue that obesity has become (2, 4). Obesity is nowadays considered to be a worldwide epidemic and has become a focus of intense research (1, 2, 4). The prevalence of the disease has been increasing in all age ranges (3). The rising prevalence of being overweight or obese leads to an increase in prevalence of diseases directly related to obesity, and to rising healthcare expenditures (3). These expenditures in the health care system are a cause of concern for governments and urge them to make extensive efforts to handle this particular health problem and its consequences (4).

In the past decades, obesity has become a major worldwide health problem (1, 3). The prevalence of obesity has been rising and is, in a lot of countries, still rising. It is estimated that around 1,2 billion adults, this is approximately 1 out of 7 of the global population, are overweight or suffer from obesity (figure 1) (2). If the trend of the last decades persist, peak obesity average-age standardized prevalence in Europe will be reached in 2037 with a prevalence level of 31% for the age group of 20-84 years (6). It is also important to note the rising prevalence in children, because of the greater impact of extreme obesity on mortality in later life amongst children compared to older adults (3). While some sources report stabilization or even decline in obesity prevalence in for example the United States of America, data from the National Health and Nutrition Survey prove the opposite. There is consensus about the increasing prevalence in developing countries (1, 3, 4). In the end, obesity is considered to be the greatest challenge of health care systems and biggest threat to worldwide health of our times (3).

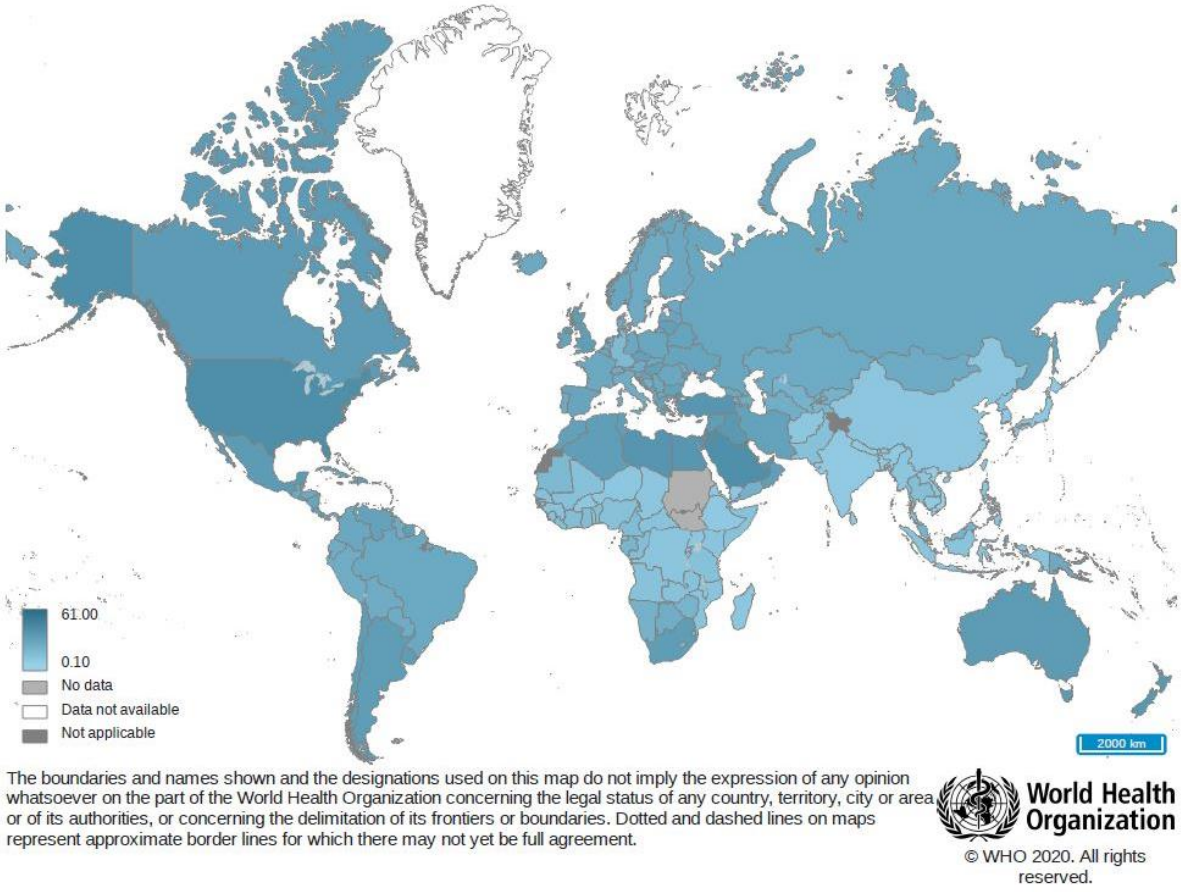

Figure 1: prevalence of obesity (BMI ≥ 30 kg/m²) among adults in 2016 (7).

1.1.2 Etiology and risks

Obesity is a multifactorial disease. This means that a complex interplay between genetic and environmental factors is at the core of the disease (4). Hereditary factors have an important role in influencing body weight; some studies suggest a 40-70% contribution of genetics in determining weight (1). On top of that, the biological variability is high (4). Certain diseases, including psychological fragility, or life-style connected factors including poor eating habits or work-related disruptions of the circadian rhythm are all environmental factors that contribute to developing overweight or obesity (2).

Obesity impacts different aspects of the human body. The extensiveness of the consequences of obesity in the human body cannot be underestimated; obesity is mostly associated with an increased risk for insulin resistance, diabetes mellitus type 2, cancers and especially cardiovascular diseases (1-5). This results in a higher risk of disability, disease and in the end, a pre-term death (2). Hypertension, atherosclerosis, hypercholesterolemia, hypertriglyceridemia, … are all contributing factors to developing cardiovascular diseases (1-3). Hyperlipidemia, heart disease, diabetes mellitus type 2 and insulin resistance are collectively covered by the term ‘metabolic syndrome’. There is no universal consensus about

the criteria defining the metabolic syndrome, but obesity and hypertension are key factors (3). Regarding the link between obesity and an increased risk of various cancer types, leptin may play a crucial role in this cascade, given it activates different pathways (phosphatidylinositol 3-kinase, protein kinase B, mitogen-activated protein kinases, signal transducer and activator of transcription 3, mammalian target of rapamycin) to promote cancer growth in presence of obesity (1, 3, 4). Furthermore, obesity has been linked with sleep apnea, gynecological problems, osteoarthritis, periodontal disease, chronic kidney disease, liver and gall bladder diseases, … (1). It also has an immense impact on life expectancy; smoking has been overtaken by obesity as the biggest cause of premature death (3). In addition, patients with obesity report a decrease in quality of life. Finally, a lot of patients experience psychological issues: they undergo discrimination and stigma, they suffer from low self-esteem, anxiety, being bullied, … (3).

A remarkable association has been discovered after observing hospital mortality rates among intensive care patients. It is drastically increased in underweight patients, but decreased in obese patients compared to normal-weight patients. Furthermore, obese patients with diabetes mellitus type 2 or cardiovascular diseases have been reported to have better survival rates than patients with the same disease but without obesity. Both observations are seen as components of the obesity paradox (1, 3). It is still a major topic of interest in scientific literature, and the underlying mechanisms are still subject of discussion. Confounders and the limitation of the BMI-tool cannot be excluded as explanations (1, 3, 4).

It is important to note that not all patients who have been categorized as ‘obese’ have the same potential fatal health risks. Up to 30% of obese patients have similar or even better health parameters (intima media thickness of the carotid artery, insulin sensitivity, visceral fat content, …) in comparison with normal-weight patients. These obese patients are considered metabolic healthy (‘metabolic healthy obese’, MHO) and are not at the same risk of developing metabolic comorbidities compared to ‘metabolic unhealthy’ patients. However, their mortality risk is still higher in comparison with normal-weight patients. That’s why MHO-patients are regarded as an intermediate-risk group in the spectrum of obesity phenotypes (3).

1.1.3 Pathophysiology

1.1.3.1 Metabolic disbalance

Obesity is characterized by an excessive amount of adipose tissue. Adipose tissue (or ‘fat tissue’) is nowadays regarded as an endocrine organ system, containing nerve tissue, connective tissue, lymphatic tissue, immune cells and especially adipocytes (2, 4). Adipocytes, also known as lipocytes or fat cells, have the function of storing (excessive) energy in the form of fat (2). The adipocytes and adipose tissue in general produce an extensive variety of

adipokines (also called ‘adipocytokines’), which have an effect on several physiological functions (2, 4, 5). In the situation of excessive energy storage, the adipose depot will expand, concomitant with a change of the endocrine and metabolic functions of the adipose tissue (2, 4). On top of that, there is an increase of resident immune cells in adipose tissue, a situation that alters the environmental immune state of the adipose tissue (2). As a result, not only the metabolic balance in the adipose tissue itself but also the homeostasis within the whole body in general can be disturbed (2, 4).

1.1.3.2 ‘The good and the bad’ adipose tissue

Adipose tissue is stored in different depots in the human body. Some of depots are filled as a healthy way to store excessive energy; others are considered a sign of metabolic imbalance. Different locations have been identified storing adipose tissue; in general, the following locations are distinguished: abdominal subcutaneous, visceral cavity, lower body (thighs, buttocks, …) and upper body (shoulders, neck, …). Fat storage in abdominal subcutaneous regions and lower body are considered ‘good fat’, contrary to storage in the visceral cavity (intra-abdominal) and upper body regions, which are seen as ‘bad fat’. Indeed, it is an excessive amount in upper body regions and visceral regions which are mostly associated with metabolic imbalances and the highest risk of related diseases. Depots at different locations show different characteristics and exhibit pro-inflammatory traits to a greater or lesser extent. This is concluded from the way cells in adipose tissue respond to cellular injury and the nature and/or number of different mediators they release. However, it is not solely the location of the adipose tissue that determines its consequences. Each adipose depot has its own characteristics, with its own metabolism, innervation, angiogenesis, … All these factors contribute in determining how depots react to their excessive growth (4).

Besides location, distinctions between types of adipose tissue have been made based on their function. In general, two main types of adipose tissue are distinguished. White adipose tissue is used as storage for lipids. These lipids are released when necessary, e.g. during a longer period of fasting. It is mainly the white adipose tissue that secretes the adipokines. On the other hand, brown adipose tissue is characterized by the strong presence of mitochondria in comparison to white adipose tissue. Brown fat serves to generate heat by burning lipids and glucose, which can result in weight loss. It is especially during the first years of an individual’s life that brown adipose tissue is significantly present (2, 4). Further research on the different types of adipocytes and their functions in immunology could help us in the understanding of obesity (2).

1.1.3.3 The immune environment in adipose tissue

As stated earlier, adipose tissue contains a wide range of different immune cell types (2, 4). It is a dynamic balance which can be altered by adipokines, produced by the adipose tissue itself (1, 2, 4, 5). In healthy individuals, low levels of pro-inflammatory immune cells are found in the adipose tissue. In a state of obesity, immune cells accumulate in the adipose tissue and shift towards a more pro-inflammatory state (2, 4). This low-grade inflammatory state is characterizing obesity (2, 5). Below, some of the key mediators of the immune environment in adipose tissue are discussed. The cells present in adipose tissue can act cell-autonomously and/or by producing cytokines, known as adipokines.

1.1.3.3.1 Macrophages

Obesity is characterized by an expansion of adipose tissue partly by an increase of macrophages. It has been suggested that macrophages represent 40-50% of the stromal cells in obese persons, while they represent only 5-10% of stromal cells in lean persons. Not only the number of macrophages differs between lean and obese persons, also the characteristics of the macrophages are different. A number of years ago, the population of macrophages were largely described by two subgroups: M1 and M2 macrophages. M1 macrophages were considered pro-inflammatory; they provided cytokines with a pro-inflammatory effect, such as interleukin 1 (IL-1), IL-6, IL-12, Tumor Necrosis Factor Alpha (TNF-α), Monocyte Chemoattractant Protein 1 (MCP-1), and reactive oxygen species like the ones arising from the action of Inducible Nitric Oxide Synthases (iNOS). In contrast, M2-macrophages were characterized based on the fact they secreted high levels of anti-inflammatory cytokines such as Transforming Growth Factor Beta (TGF-β), IL-4, IL-10, IL-13 and blocked iNOS-activity; resulting in an anti-inflammatory response. The environment, with all its cyto- and chemokines, determined the net phenotype of the macrophages (2, 4, 8).

This M1-M2 model was recently considered outdated. Today, researches acknowledge that there is a wide spectrum of macrophages which cannot be simply divided in only two subtypes. Metabolic phenotyping, surface markers and RNA sequencing are necessary to identify macrophages in this wide spectrum. When the outdated two-dimensional model was used, it was believed that there is an imbalance leaning towards an excess of so-called M1-macrophages, favoring an enhanced secretion of pro-inflammatory cytokines. This model has been criticized by some findings, among others based on the finding that human macrophages in adipose tissue hardly express markers that allow a distinction between M1 and M2-macrophages as in the classic concept. The most recent viewpoint supports that the obese environment accommodates a massive presence of macrophages in adipose tissue, enhancing lipid metabolism, lysosomal functioning and cell adhesion (2, 4, 8).

1.1.3.3.2 Neutrophils

Extrapolating studies using obese mice suggest an increase of neutrophils in adipose tissue may also take place in obese people. This leads to a decreased insulin sensitivity and to glucose intolerance. On top of that, these neutrophils show a higher secretion of superoxides, which have a role in activating macrophages towards a pro-inflammatory state. It is believed that bariatric surgery can decrease neutrophil levels (2).

1.1.3.3.3 T-cells

T-cells incorporate a wide variety of cells. In general, the population of T-cells can be divided in 3 subpopulations: CD4 T-cells, CD8 T-cells and Natural Killer (NK) T-cells.

CD4 T-cells differentiate into different types of T-helper cells after recognizing antigens in combination with MHC class II. There is a wide range of T-helper cells, with every type of T-helper cells secreting different Interleukins. Just like CD4 T-cells, CD8 T-cells recognize antigenic peptides but in combination with MHC Class I. Although they also release cytokines, the most important function of CD8 T-cells is cytolysis of target cells. Because of this, they are also called 'effector T-cells'. NK-cells form a bridge between adaptive and innate immune responses and can act as effector cells, just like CD8 T-cells (2).

In obese patients, CD8 T-cells in adipose tissue are increased, whilst CD4 T-cells and especially Regulatory T-cells (Treg-cells) are decreased. The increase in CD8 T-cells is linked with an increased recruitment and activation of macrophages. CD4 T-cells differentiate into various types of T-helper cells, depending on the immune environment. In general, Type 2 T-cells (Th2 cells) and Treg-cells are considered to mediate an anti-inflammatory response in healthy patients, while Type 1 T-cells (Th1 cells) and Type 17 T-cells (Th17 cells) cells contribute to an enhanced inflammatory state. It is no surprise that increased levels of Th1 and Th17 cells are observed in obese patients, and consequently a rise in IL-17A and IL-22 serum levels. This is accompanied by an increase of IL-17- and IL-22-receptors on macrophages, making those cells even more sensitive to these cytokines. This process has been linked to a limited glucose uptake, which eventually leads to metabolic dysfunction. Another observation in obese patients is the decrease in circulating Treg-cells. As such, the anti-inflammatory effect of Treg-cells is decreased in comparison with normal weight individuals (2, 4, 5).

1.1.3.3.4 Other mediators

In adipose tissue, a wide variety of other types of immune cells, such as for instance B-cells, is also present. Obesity can alter the balance of these immune cells in many ways. In obesity, B-cells are more often transformed into mature B-cells which infiltrate in adipose tissue, contributing to the inflammatory response, instead of transforming into Regulatory B-cells that mediate a net anti-inflammatory effect. Other immune cells playing a role in creating a more

pro-inflammatory state in adipose tissue among obese patients are for example basophiles, mast cells, mucosal-associated invariant T-cells, dendritic cells and myeloid-derived suppressor cells (2).

1.1.3.4 Adipokines

White adipose tissue secretes soluble signaling factors named ‘adipokines’ or ‘adipocytokines’. There is still discussion about what exactly the terminology refers to, but in general, they are considered mediators produced by adipose tissue. Adipokines regulate different physiological processes, such as cell growth, inflammation and appetite (2, 4, 5). Cytokines produced by immune cells in adipose tissue are also regarded as adipokines, however, sometimes they are referred to as ‘pseudo-adipokines’ (4, 5). The majority of adipokines have a pro-inflammatory effect and have an important role in the development of some comorbidities. It is believed that white adipose tissue secretes more than 50 different adipokines (5). In this thesis only the adipokines that are most frequently documented, are highlighted.

1.1.3.4.1 Leptin

Leptin was the first adipokine to be identified (5). It is produced by adipocytes, but not exclusively (4). Leptin is released in a state of satiation and acts on the hypothalamus. This results in a suppression of hunger maintaining a healthy state of homeostasis (4, 5). Leptin levels are directly correlated with adipocyte size and body adipose mass (5). This helps to understand why leptin levels are higher in obese patients. Leptin has pro-inflammatory effects as well, reached by different pathways (1, 2, 4, 5). It activates macrophages, stimulating them to secrete pro-inflammatory cytokines, and CD4 T-cells, stimulating them to become Th1 cells and leading to secretion of pro-inflammatory cytokines. On top of that, leptin inhibits the proliferation of Treg-cells, promotes the function of neutrophils, … (2, 4, 5). In short, obesity leads to increased levels of leptin which may be consistent with a more pro-inflammatory state (5).

1.1.3.4.2 Adiponectin

Adiponectin is primarily released by adipocytes in white adipose tissue (4, 5). In contrast to most adipokines, adiponectin has an inverse correlation with an individual’s weight; it is decreased in obese patients (2, 5). Adiponectin has a wide variety of functions in the body, however, improving insulin sensitivity is one of its prime functions (4, 5). Contrary to leptin, adiponectin is predominantly associated with an anti-inflammatory effect. This is achieved by its effects on endothelial cells of blood vessels. These effects include reduced adhesion of monocytes to endothelial cells by limiting the expression of TNF-α-induced adhesion molecules. Adiponectin also inhibits proliferation, maturation and activity of macrophages and inhibits their secretion of pro-inflammatory cytokines, such as TNF-α and IL-6. A decrease in

adiponectin levels is associated with different obesity-linked diseases, including heart diseases and hypertension. However, also pro-inflammatory effects of adiponectin have been described, especially in autoimmune diseases (5).

1.1.3.4.3 Resistin

Resistin has been identified as an adipokine with higher levels in obese patients. More research is still necessary, but researchers consider resistin an adipokine with pro-inflammatory effects and able to promote insulin resistance. The underlying molecular mechanism is still subject of research, but studies suggest a role for activation of Suppressor of Cytokine Signaling 3 (SOCS3), leading to the suppression of insulin-mediated signaling (2, 4, 5). Resistin has a similarly mechanism of action to leptin; it stimulates macrophages to secrete pro-inflammatory cytokines, such as TNF-α and IL-6 contributing to a net pro-inflammatory effect (4, 5). It is believed that resistin has effects on vascular endothelial cells as well, again with a pro-inflammatory character, contributing to the development of atherogenesis (5).

1.1.3.4.4 TNF-α

TNF-α is one of the most broadly researched cytokines and the link with inflammation has been extensively studied. Different drugs targeting TNF-α have been developed and used as treatment in diseases characterized by an inflammatory state, e.g. Rheumatoid Arthritis (RA) (see below 1.2.5.2.1.3) (4, 9). Sometimes considered a 'pseudo-adipokine', TNF-α is still a signaling factor produced by immune cells in adipose tissue (4). Produced amongst others by macrophages, there is a significant increase in secretion in obese patients. This increase was discovered more than twenty years ago and then suggested to be associated with insulin resistance because of obesity; meanwhile, this has been proven in different studies (2). TNF-α contributes to inflamed of adipose tissue in different ways. Together with IL-6, it is believed to be one of the cytokines contributing the most to the inflammation seen in obesity (2, 4, 5).

1.1.3.4.5 Other adipokines

As mentioned earlier, more than 50 adipokines have been identified. This includes Visfatin, Omentin, Vaspin, Lipocalin-2, …(2, 4). It is not the aim of the thesis to give an overview of all existing adipokines. In this perspective, we refer to other studies to have a general view of all acting adipokines. However, further investigation of adipokines is necessary to disentangle the different mechanisms linking obesity and inflammation (5). After all, it is clear that adipokines are secreted by adipocytes and can have important pro-inflammatory properties, contributing to the low-grade inflammation state characteristic of obesity (2, 5).

1.1.4 Diagnosis and follow-up

Different tools are used to evaluate the weight of individuals and to make a classification. The assessment of individuals is important to estimate the associated health risks and, accordingly, to be able to apply individualized treatment. At the moment, the most popular tool to classify overweight and obesity is BMI (3-5). BMI stands for Body Mass Index and can be calculated by using the following formula: $\frac{\text{weight (in kilograms)}}{\text{height (in meters)} * \text{height (in meters)}}$ or $\frac{\text{weight (in pounds)} * 703}{\text{height (in inches)} * \text{height (in inches)}}$ (4, 5). The resulting number is one's BMI and expresses the weight-for-height ratio of the individual (4). Based on BMI, individuals are subdivided in different categories (table 1) (3-5).

Table 1: definitions of underweight, normal weight, overweight and obesity based on different BMI cut-off values (3).

Categories	BMI cut-offs (kg/m²)
Underweight	< 18,5
Normal weight	18,5 – 24,9
Overweight	25 – 29,9
Obesity Class 1	30 – 34,9
Obesity Class 2	35 – 39,9
Obesity Class 3	≥ 40

These cut-offs still remain valid when examining children, according to the International Obesity Task Force (IOTF) (3).

Morbid obesity is defined as obesity class 3 or obesity class 2 in combination with significant co-morbidities related to the obesity (3).

BMI has become the international standard of measuring and classifying overweight and obesity (3, 4). It has a central role in the guidelines reported by the World Health Organization and has turned use of relative weight as a measure to evaluate patients obsolete (4). Thanks to its simplicity to calculate, its applicability at any moment to every population, and its correlation with a lot of health issues, it has also become the preferred method by researchers (1, 3, 4). However, its straightforwardness is also subject of criticism (5). It only represents a score achieved by a simple calculation, which limits its value (4). Too often, it does not precisely reflect the body composition of a person (3, 4). For example, BMI does not convey information on the proportion of fat of the body weight, and where it is distributed; a factor that obviously affects someone's health (3). BMI does not differentiate between muscle mass and adiposity mass, while both show totally different underlying molecular mechanisms (4). These limitations are considered by inclusion of 'normal weight obesity'. This has been defined as a person classified by the BMI-cut-offs as a normal weight individual, despite having a high body

fat percentage and thus resulting in an increased state of metabolic imbalance and higher risk of developing metabolic syndrome, cardiovascular health issues and consequently mortality (4). The concept of 'obesity paradox' is also showing the limits of BMI. This concept refers to findings where having a higher BMI was associated with less (severe) metabolic diseases, less mortality and better outcomes in general (1, 3, 4). This can be considered proof that BMI and the linked diagnosis of obesity do not always relate with co-morbidities associated with disproportionate adipose tissue disposition (4).

Alternatives have been proposed to replace BMI or to be added to BMI to get a better evaluation of someone's health risks based on weight. Measuring the Waist Circumference (WC) is an alternative (1, 3, 4). Different authors suggest WC as tool to assess normal weight and overweight persons. Some have proven that it was one of the best predictive tools to analyze adipose tissue mass situated intraperitoneal and posterior subcutaneous. Combined with BMI, WC is strongly associated with metabolic comorbidities (4). Another proposed measure is inclusion of the waist-to-height ratio. It has been related with a significant increase in cardiovascular risk, especially with children (3). In the same class, waist-to-hip ratio has been reported as another additional tool (1, 3). Combining the height and hip circumference, body adiposity index has been successfully used as predictor of obesity (3). While some authors put emphasis on the association with leptin levels, other studies rated WC and waist-to-hip ratio higher (3). Building on BMI, the calculated BMI-numbers can be transformed into BMI percentiles by using growth charts, showing the relative position among a given population (3).

As mentioned earlier, the ideal tool would consider individuals as obese based on characteristics of adiposity instead of weight (4). After all, it is the promotion of inflammation by adiposity which links obesity to metabolic diseases (4). The distinction between good and bad adiposity, the amount of adiposity and the distribution of enhanced depots, are all factors that need to be evaluated to have an accurate adiposity-based risk stratification to assess someone's risk of developing co-morbidities linked to obesity (4). A way to analyze the adiposity in an individual is using the dual energy X-ray absorptiometry (DEXA) (1, 4). This analysis can give the percentage of body fat in a human body. There are different studies which show a discrepancy between BMI and the body fat index calculated by DEXA. Those studies provide evidence supporting the critical questions raised about the inaccurate link between BMI, adiposity tissue and health outcomes (4). Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) are other ways of achieving a-more-in-depth analysis of fat depots in the human body. It is important to highlight the disadvantages of CT: due to the massive use of ionizing radiation, pregnant women and children are to be avoided as a population for which CT can be used (1). DEXA, CT and MRI deliver more accurate results

about the composition and location of adipose deposition, and consequently a more precise correlation to the level of co-morbidities risks (1, 4). While it is broadly used by researchers, it is often too expensive and cumbersome to use in clinical practice and difficult to execute with patients having BMI's of 35 or higher (1, 4).

In the end, despite its limitations, BMI remains the most popular tool to be used for the stratification of risk in obese populations (1, 3, 4). However, being aware of its limitations and/or combining it with other tools remains necessary to evaluate a person's risk of developing metabolic abnormalities due to overweight or abnormal adiposity (1, 3, 4).

1.1.5 Treatment

The first-line approach to obesity is to raise awareness about the risks of the disease and inform the patient about the need to live a healthier life, and encourage the patient to adjust to a life-style resulting in weight loss and a loss of adipose tissue. Ideally, this is used as a preventive approach for patients being at risk of becoming overweight. There is more and more focus on interventions targeting communities and interventions being integrated in the policy of preventive health care. New approaches using technology such as smartphones, apps and social networking are subject of research and can impact public health. In addition to lifestyle changes, some medications are used to treat obesity such as Orlistat and Lorcaserin. However, the success rate is limited and the side effects are extensive. Bariatric surgery has known an increase in popularity in the last decades due to its improvement in effectiveness and safety. It can be used for patients having a BMI above 40 or above 35 in combination with some comorbidities e.g. type 2 diabetes mellitus or cardiovascular diseases. However, the risks of the surgery are not to be underestimated and the surgery demands patients to follow a strict diet. After all, preventive and lifestyle interventions are the best method to put an end to the growing obesity epidemic (1). Encouragingly, it has been proven that diet and exercises effectively decrease the inflammatory state. It decreases the levels of pro-inflammatory cytokines as IL-6, TNF-α and decreases C-reactive protein (CRP) levels (2).

1.2 Rheumatoid Arthritis

1.2.1 Definition and epidemiology

Rheumatoid arthritis (RA) is a chronic, immune-mediated inflammatory disease (9-11). RA affects the joints, especially small joints of hands and feet, leading to joint damage and bone destruction (10, 11). This can lead to severe disability (10). The worldwide prevalence of RA is about 5-10 per 1000 adults worldwide (10-12). People between 65 and 74 years show the highest incidence of RA (10, 11). Women are affected 2 to 3 times more than men at any age (10, 11). Besides women, smokers and people with a family history of RA are more at risk to develop the disease (9).

1.2.2 Symptoms and evolution

The first symptoms are often pain or swelling of joints, typically the smaller joints of hands or feet, such as the wrist and metacarpophalangeal joints (figure 2). RA however rarely affects the distal interphalangeal joints. In addition, larger joints can be affected, including shoulder, elbow, knee and ankle joints. Psoriatic arthritis, a variant of spondyloarthritis characterized by inflammatory peripheral arthritis and skin psoriasis, can also induce swelling of the fingers, but this involves the whole digit, whilst in RA the swelling is more concentrated around the joint. Swelling of joints is also observed in osteoarthritis (the most frequent type of arthritis characterized as a dynamic, regenerative, remodeling condition with mechanical pain in joints), but in this case swellings are considered to be hard because they originate from swollen bones, whilst the swelling in RA is typically soft because there the swelling is located in the synovium (10, 13, 14). RA should always be considered as diagnosis when a patient suffers from at least one swollen joint that does not readily heal, without explanation or any other diagnosis (9). Besides swollen joints, morning stiffness possibly lasting multiple hours is also seen as one of the possible symptoms in early stages of RA (10, 14). This can be accompanied by non-specific symptoms including a flu-like feeling and weakness (14).

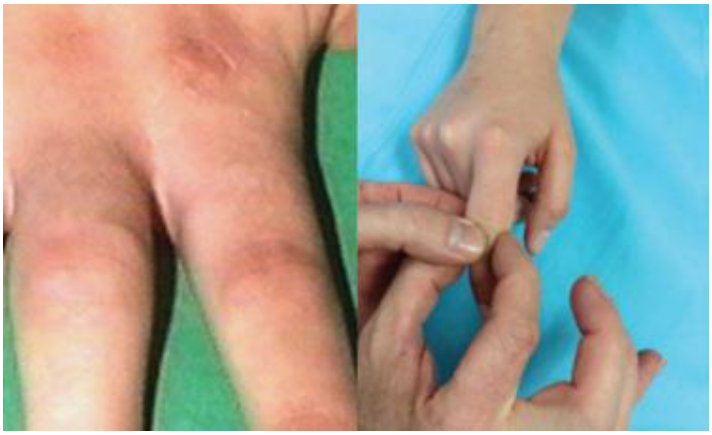

Figure 2: picture of swelling of a proximal interphalangeal joint of the finger. Swelling of joints is one of the first clinical symptoms of RA (14).

Treating RA rapidly and adequately when these early-onset signs and symptoms are observed is crucial (10, 14). Otherwise, RA progresses into a more destructive disease with disabling symptoms (10). The progression of RA leads to extra-articular manifestations, such as rheumatoid vasculitis and, more frequently, rheumatoid nodules. Other manifestations include keratoconjunctivitis, episcleritis, other auto-immune diseases affecting the thyroid gland, and lung diseases (9, 10, 14). The chronic inflammatory state of RA also impacts the cardiovascular system. Cardiovascular diseases are frequent complications of RA and an important cause of death in RA-patients. Therefore, cardiovascular risks in these patients should be identified and tackled if possible. On top of that, other cardiovascular risks factors such as obesity and smoking decrease the chance of achieving clinical remission (10, 14).

The specific antibodies that play an important role in the pathophysiology of RA, develop before any of the symptoms are established. Depending on which type of antibodies develop in the body, the lag time between the developed antibodies and the symptoms differ from months to 10 years. Hence, the typical debilitating symptoms of RA only occur in a more advanced state of the disease (10).

New findings on the pathogenesis of RA have led to the development of new drugs which have drastically improved the lives of RA-patients (10, 14). In the end, 75 to 80% of all patients can achieve low disease activity or remission. They can participate normally in society, with opportunities to go to work and live a social life. Their life expectancy is not changed by RA. However, 20 to 25% of all patients do not reach low disease activity. Among those, the disease has a substantial effect on their lives. Increased mortality rates are seen in patients with persistent high disease activity (10).

1.2.3 Pathophysiology

In the past decades, progression has been made in understanding the mechanisms of the pathophysiology of RA. This can help developing more effective treatment strategies (10).

The primary site of inflammation in RA is the synovium. Synovium is a membrane layer surrounding the articular cavity in a joint and plays an important role in secreting components of the synovial fluid (10).

The pathophysiology of RA starts with the pre-articular phase (11). In this early disease stage, there is almost no damage to cartilage or bone (10). This phase is characterized by developing antibodies against components of the own body (autoantigens) (11). Some auto-antibodies, the anti-citrullinated peptide antibodies/anti-cyclic citrullinated peptide antibodies (ACPAs/anti-CCPs), are directed against citrulline, which is a variant of the amino acid arginine, and some are directed against the Fc-portion of IgG, called Rheumatoid Factor (RF) (10, 11). These

antibodies activate dendritic cells which stimulate the growth and differentiation of T-cells, especially Th1-cells, Th17-cells and T-reg cells (11). These cells infiltrate the synovial membrane together with B-cells and monocytes (10). Rheumatoid synovium is also characterized by the migration of RA synovial fibroblasts (RASFs), aggressive cells invading cartilage and synovium. RASFs secrete cytokines and chemokines stimulating the further migration of immune cells into hyperplastic synovium, leading to increasing synovitis (15, 16). Following the infiltration of immune cells in the synovial membrane, this membrane expands and is called “pannus” (10). The infiltrated immune cells trigger the release of cytokines which contribute to the phase of the disease characterized by joints and bone destruction resulting in swelling and pain. The pro-inflammatory cytokines induce different molecules like prostaglandins and matrix metalloproteinases (MMPs). They also stimulate the release of receptor activator of nuclear factor kappa-B ligand (RANKL) which binds its receptor, receptor activator of nuclear factor kappa-B (RANK) on precursor osteoclast cells. This leads to the differentiation of osteoclast precursors to osteoclasts, of which the normal function is to degrade bone, hence an overstimulation leads to bone damage (10, 11). The elevated levels of MMPs contribute to bone degradation and serve as biomarkers for the ongoing destruction of cartilage. In the end, the process accelerates when bone joints start rubbing against each other as a result from the degradation of cartilage (11).

The cause for triggering these processes in RA is still unknown. Different genetic and environmental factors are identified in contributing to RA. These genetic factors include some Human Leukocyte Antigen (HLA) class II antigens which have stronger associations with RA. There is a wide variety of environmental factors which induce a higher risk of developing RA. This includes smoking, some viral infections, specific characteristics of the microbiome of the gut and periodontitis (10, 11).

1.2.4 Diagnosis and follow-up

As mentioned earlier, diagnosis of RA in an early stage is crucial. Treatment started in an early stage before joints are irreversibly damaged can prevent progression of joint damage in 75-90% of the patients (9, 10). Unfortunately, it is still a challenge to detect developing RA expeditiously (14). There are no universal diagnostic criteria to diagnose a patient with RA (10). However, different scoring systems have been developed. While they were not developed with the aim of being diagnostic, they help the physician to identify possible new RA cases and reveal different aspects and considerations when diagnosing a RA-patient (10, 14).

The European League Against Rheumatism (EULAR) 2010 classification is a scoring system consisting of different criteria helping to assess a patient with symptoms of RA. These symptoms are frequently seen in early stages of RA and differentiate from other forms of

arthritis (10, 11, 14). Four general criteria can be distinguished. The **first** criterion concerns the number of damaged joints, going from one large joint to multiple small joints. The assessment of joint involvement can be evaluated by physical examination or by radiological investigations (10, 11). Plain radiography and Magnetic Resonance Imaging (MRI) are the most important tools to identify erosions in joints. However, plain radiography is quite insensitive to detect the early bone lesions and MRI is not always available (11). Ultrasound and specifically Doppler Ultrasound are often used to detect active joints, but there is discussion about the benefit of this technique to detect RA in early stages (11, 14).

The **second** criterion by EULAR concerns serology results specific for RA. Different types of anti-immunoglobulin tests are used in diagnosing RA, all with their own advantages and disadvantages. By EULAR, score points have been assigned to a positive RF and/or ACPA (10, 11). The RF-antibodies, which are directed against the Fc-portion of IgG, are usually tested by measuring the IgM levels. Although the result can be false positive or negative, it is still considered a good marker with good sensitivity and specificity. RF can be measured in serum as well by nephelometry (measuring the antigen-antibody agglutination reaction by light beam strike wavelength) which is more accurate but at a higher cost. Besides RF, anti-CCP antibodies also called ACPAs are another type of useful markers in blood and, because of their higher specificity over RF, often used as a prognostic and follow-up tool. In some cases anti-dsDNA is measured in serum but this is less sensitive and specific as compared to the other markers (11).

The **third** criterion is also relying on serology to characterize possible RA cases, more precisely the evaluation of the inflammatory state by non-specific tests. These tests include acute phase proteins (CRP), erythrocyte sedimentation rate and plasma viscosity. Positive scoring of those tests are consistent with RA diagnosis (9-11). Other non-specific tests which can aid to the diagnosis of RA are white blood cell counts, thrombocyte count, serum hemoglobin and iron levels. An increase in white blood cell counts and a decrease of serum hemoglobin, iron levels and thrombocyte count fits in the expected inflammatory state of the patient (11).

The **fourth** criterion on which score points are given to a patient for the evaluation of possible RA-diagnosis, is the number of weeks the patient already has symptoms typically for RA. When symptoms are present for 6 weeks or more, a score point is added (10, 11). All score points of the four criteria are added together. A patient with 6 or more points can be considered a patient with RA (10).

The EULAR-consortium indicates useful investigations to help identifying patients with (early onset) RA. Other scores are often used in the follow-up of patients and their response to

treatment. These instruments include the Clinical Disease Activity Index (CDAI), the Disease Activity Score using 28 joint counts (DAS28) and the Simplified Disease Activity Index (SDAI) (9, 10, 17). They try to summarize the patient's status of disease in a single score and can help in evaluating the effect of treatment. The CDAI is the simplest score to perform, consisting of four criteria: the patient global assessment and the evaluator global assessment, both using a visual analogue scale, and the amount of tender and swollen joints. The numerical summation ranges from 0 (best score) to 76 (worst score). The CDAI and SDAI are the best tools to evaluate clinical remission of the disease (10). However, while it is crucial to notice the benefit of these instruments to identify new patients, to follow the state of disease activity in a RA-patient and to identify remission, it is clear that these instruments cannot be used solely to diagnose RA and any score always need to be linked to the symptoms of a patient (10, 14).

1.2.5 Treatment

1.2.5.1 General principles

Patients diagnosed with RA had a poor prognosis until the 1990s because of insufficient knowledge about the disease and the lack of good medication (14). However, treatment for patients has significantly improved in recent years. This is due to new insights in the pathogenesis of RA, which formed the basis for the development of new medications (10, 14). There is a broad consensus that the aim of treatment is remission of the disease in patients, and if this cannot be successfully achieved, that the disease activity should be lowered as much as possible (14, 18). Remission and lower disease activity can be evaluated by earlier mentioned techniques like the SDAI- and CDAI-indexes (18). It is important to note that even if low disease activity is reached, RA remains a chronic disease which cannot be cured in most cases and which usually needs lifelong intake of medication (10, 18). This leads to a significant part of healthcare expenditures for both patient and society, an aspect which also should be considered when managing a treatment strategy. The rheumatologist is the specialist in treating patients with RA and has a central role in choosing treatment strategies. However, these decisions should always be a shared decision between the rheumatologist and the patient. Both actors need to be aware that achieving the therapeutic goal is frequently a long and difficult process consisting of frequent changes in the treatment strategy (18).

1.2.5.2 Treatment options

Drug therapy is the main focus of treatment. There are different non-pharmacological treatments like lifestyle changes, surgery, and occupational and physical therapy which can be considered for every patient and especially when drug therapy fails. However, drugs are necessary to treat the inherent inflammatory component within the RA disease (11, 14). At this moment, the most frequently used available drugs for RA-patients are **non-steroidal anti-inflammatory drugs (NSAIDs)**, **disease-modifying anti-rheumatic drugs (DMARDs)** and

glucocorticoids. Especially the latter classes of drugs are crucial in the control of RA (11, 14, 18).

1.2.5.2.1 DMARDs

While NSAIDs solely have an impact on symptoms, a DMARD is by definition a drug interfering with symptoms, physical function and progression of joint damage (10). DMARDs are considered cornerstones of RA treatment and should be initiated as soon as possible in new patients (10, 11, 14, 18). Two big classes of DMARDs are distinguished. There are DMARDs with a synthetic origin, called the synthetic DMARDs (sDMARDs), which are smaller chemical molecules to be taken orally, and DMARDs with a biological origin, the so called biological DMARDs (bDMARDs) which need to be taken parenterally (10, 11, 14). The sDMARDs are divided in targeted synthetic DMARDs (tsDMARDs) and conventional synthetic DMARDs (csDMARDs). The latter class contains drug against RA recognized since decades and has a more general therapeutic effect, while tsDMARDs interfere with well-known molecules and benefit from a better understanding of the pathogenesis (10).

1.2.5.2.1.1 csDMARDs

Methotrexate (MTX) is considered to be the most important and frequently used csDMARD (10, 11, 18). One of its main effects is the decrease of adenosine levels which leads to less activation of inflammatory pathways. Different other mechanisms are reported, e.g. the irreversible inhibition of the enzyme dihydrofolatereductase which converts dihydrofolate to tetrahydrofolate. The latter mechanism isn't considered to have a crucial role in the treatment of RA, but it explains why folic acid supplementation is necessary with patients taking MTX (10, 11, 18). However, other action mechanisms of MTX are considered to be more important in RA. MTX improves the disease in 25-40% of the patients in monotherapy, and in almost 50% of patients when combined with glucocorticoids (see below 1.2.5.2.2) (10). On top of that, MTX can be combined with different other treatment options for RA (18). Alternatives for MTX include leflunomide and sulfasalazine (10, 11, 14, 18). However, these alternatives are only considered in case of contra-indications for the intake of MTX or in case of early intolerance (14, 18). The adverse effects of csDMARDs are well known and similar between different agents; they include hair loss, fatigue, nausea, cytopenia, stomatitis and hepatoxicity (10, 11, 14).

1.2.5.2.1.2 tsDMARDs

tsDMARDs comprise well-known small chemical molecules that target signaling pathways known to be over-active in RA. Drugs focusing on inhibiting Janus Kinases (JAKs)-pathways are now commonly used and have led to a breakthrough in the treatment of RA (14). JAKs are enzymes playing an important role in transferring signals from cytokines to intracellular

responses (10). Experiments have proven the safety of this drug, but cytopenia, elevated cholesterol levels and gastrointestinal problems are described as side-effects (14). Thrombo-embolic events can be caused by JAK-inhibitors and patients at risk for these events should be treated differently or with caution (18).

1.2.5.2.1.3 bDMARDs

bDMARDs focus on specific elements of the immune response. At this moment, drugs focusing on B- and T-lymphocytes and pro-inflammatory cytokines (IL-6, TNF) are used. bDMARDs have extensively improved the outcome of those patients for which the first line treatment (e.g. MTX) was not successful (11). Anti-TNF drugs are widely used. These drugs block TNF-α, which is a cytokine expressed on macrophages and which can increase the inflammatory state by binding to its receptor, in different ways (4, 9). As a downside, enhanced (risk of) infections can be caused by both tsDMARDs as bDMARDs. Therefore, screenings for latent tuberculosis and vaccinations are needed to guarantee the safety of the patient (9-11, 14, 18). On top of that, anti-TNF drugs should be handled carefully in patients with heart failure. Whilst both congestive heart failure as RA are characterized by higher levels of TNF-α, the source and function of TNF-α differs between both diseases; synthetized TNF-α in congestive heart failure may play a protective role in the protection of the failing heart. More research is necessary to develop clear recommendations about TNF-blockers in RA-patients with heart failure (9, 19).

1.2.5.2.2 Glucocorticoids

Glucocorticoids are steroid hormones acting as anti-inflammatory and immunosuppressive drugs. They can quickly delay the progression of RA (11, 12). They are often used as therapy in addition to standard DMARD's to reduce the progression of bone erosion. They should solely be used for short-term periods because of their wide range of side-effects upon chronic use (10-12, 14, 17). These may include the development of hyperglycemia, osteoporosis, gastrointestinal effects, infections, cardiovascular diseases, psychosis and other neurological disorders (12, 17).

1.2.5.3 Treatment strategy

When RA is diagnosed, or when a patient is identified as at risk of having or developing RA, treatment with DMARDs and more specifically MTX, should be started as soon as possible (9, 10, 14, 17, 18). MTX should be combined with glucocorticoids at the start of treating a patient with early-state RA (9, 10, 17). Rheumatologists are not obliged by EULAR-recommendations to use glucocorticoids, but following those recommendations, glucocorticoids should be considered (14, 18). After 3 to 6 months, the effect of therapy needs to be evaluated and glucocorticoids should be tapered to prevent side-effects (10, 18). Success rates differ between study reports: Wasserman et al. (9) states that 3 out of 4 patients undergo remission

after 16 weeks of the combination therapy, whereas Aletaha et al. (10) state that more than 50% of the patients failed to reach their treatment goal after the first course of DMARD. When this is the case, the presence of poor prognostic factors with the patient (e.g. high disease activity, presence of auto-antibodies, …) should be evaluated (10, 14, 18). If present, a conventional DMARD should be combined with a tsDMARD or a bDARMD, preferring bDMARD above tsDARMD. If the treatment goal still is not reached, the used tsDMARD of bDMARD should be replaced by another one (10, 14, 18). If the patient does not have any negative prognostic factors, another csDMARD instead of MTX can be given (18).

When remission is achieved, tapering drugs should be considered. Glucocorticoids should always be tapered after several months of intake (10, 11, 14, 17, 18). When combining a csDMARD with a bDMARD or tsDMARD and the patient has been in remission for several months, tapering bDMARD or tsDMARD is recommended (10, 14, 18). If the patient is in sustained remission after months of treatment with solely a csDMARD, the drug should also be tapered (14, 18). However, tapering can be accompanied by flares and should thus be followed up carefully (18). Patients must be aware that RA is a chronic disease and drug-free treatment can almost never be achieved; when the efficacy of a drug has been proven for a particular patient, tapering should be considered but the therapy should not be stopped (14, 18).

1.3 Research hypothesis

As mentioned above, both obesity and RA are diseases linked with an inflammatory state in the human body. Therefore, it can be assumed that both diseases can influence each other. Exploring the impact of obesity on the progression of RA, with special attention to the underlying molecular mechanisms, is the main objective of this thesis. This literature research will be achieved by collecting and analyzing relevant articles from different databases.

2. Methodology

Before starting the search process for articles in databases, general information about the topics obesity and RA was acquired. Advice by my promoter, courses given during my curriculum and the world wide web were all useful to have a first general insight into the relevant topics. The subject was analyzed by a PICO-framework to develop a clear view on the research search strategy.

The search for topic relevant articles was performed using different scientific databases. PubMed was the first database to be used. Different Medical Subject Headings (MeSH) terms were used in different combinations to achieve fitting results. In general, four different clusters were combined in the search details. First, the concepts of obesity and RA were linked to each other. However, when the observation was made that the resulting articles insufficiently captured the impact of obesity on RA, a third concept was introduced to link previous concepts. Inflammation had been identified in the earlier performed general research as a crucial element in both diseases and was therefore used as link. To gather data concerning (mice) experiments, a fourth concept was introduced and added to the search builder.

The Embase database was consulted in the aim of not missing any articles which were not available in the PubMed database (e.g. articles not included in MEDLINE). The same methodology was applied in the form of Emtree-terms (table 2). It should be noted that different ways of combining and expressing those concepts have been used. In addition, some articles have been found by the 'similar articles'-function in PubMed. At some point, different terms were used singularly and not in combination to find relevant (review-)articles for introducing the subject.

Google Scholar and Web of Science were other databases which have been consulted during the research process. The same terms as in PubMed and Embase have been used. However, the research process was less extensively performed in comparison to the PubMed- and Embase-databases.

In total, 197 articles were selected and put into EndNote. This selection was made by filtering articles of the last 10 years and written in the English language. These selected articles were analyzed deeper, including identifying the availability of the articles online and speed-reading the results. After consultation with my promoter, the final decision selection of articles was made. It should be noted that different articles were added during the writing process, both during the writing process of the introduction of RA to extend the information about different specific topics and during the writing process of molecular mechanisms in the results section to further explore different specific topics. The originally selected EULAR-recommendations of 2016 were replaced by these of 2019 (17).

Table 2: overview of the used MeSH terms and Emtree Terms categorized by topic in respectively the PubMed- and Embase-database.

	PubMed – MeSH terms	Embase – Emtree Terms
Rheumatoid Arthritis	"Arthritis, Rheumatoid"[Mesh:NoExp]	'rheumatoid arthritis'/mj
Obesity	"Obesity"[Mesh:noexp] OR "Body Mass Index"[Mesh] OR "Adiposity"[Mesh] OR "Overweight"[Mesh] OR "Hypertriglyceridemic Waist"[Mesh] OR "Lipid Accumulation Product"[Mesh] OR "Obesity Management"[Mesh] OR "Metabolic Syndrome"[Mesh] OR "Sedentary Behavior"[Mesh] OR "Body Weight"[Mesh] OR "Exercise"[Mesh] OR "Nutrition Surveys"[Mesh] OR "Hyperlipidemias"[Mesh] OR "Adipose Tissue"[Mesh] OR "Dietary Fats"[Mesh] OR "Diet"[Mesh]	'obesity'/exp/mj OR 'body mass'/exp/mj OR 'adipose tissue'/exp/mj OR 'obese patient'/exp/mj
Link between RA and Obesity (e.g. inflammation)	"Inflammation Mediators"[Mesh] OR "Cortisone"[Mesh] OR "Anti-Inflammatory Agents" [Pharmacological Action] OR "Anti-Inflammatory Agents"[Mesh] OR "Glucocorticoids"[Mesh] OR "Disease Progression"[Mesh] OR "Prognosis"[Mesh] OR "Severity of Illness Index"[Mesh] OR "Risk Factors"[Mesh] OR "Remission Induction"[Mesh] OR "Obesity/complications"[Mesh]	'disease exacerbation'/exp OR 'prognosis'/exp OR 'disease severity'/exp OR 'severity of illness index'/exp OR 'disability severity'/exp OR 'risk factor'/exp OR 'inflammation'/exp
Experiment (mice, animals)	"Animals, Laboratory"[Mesh] OR "Disease Models, Animal"[Mesh] OR "Mice, Obese"[Mesh] OR "Mice, Mutant Strains"[Mesh] OR "Models, Biological"[Mesh] OR "Signal Transduction"[Mesh] OR "Arthritis, Experimental"[Mesh] OR "Mice"[Mesh] OR "Physical Conditioning, Animal"[Mesh]	'experimental mouse'/exp OR 'molecular mechanics'/exp OR 'molecular biology'/exp

3. Results

In the first results section, an overview will be given of clinical studies evaluating the impact of obesity on RA. In the second results section, mechanisms of pathophysiology will be brought forward to clarify findings of the first section and to meet the aim of this paper.

3.1 Clinical parameters

Many clinicians have examined the impact of obesity on the progression of RA. Some of the research has been performed by longitudinal studies, others have performed cross-sectional studies. It is clear that RA cannot simply be diagnosed by only one parameter; different aspects define the entirety of the disease. Therefore, although different studies deal with different features of or mechanisms behind RA, all studies comprised here have been selected with the aim to expose the impact of obesity on the disease. First, the most frequently used tools to establish the disease severity of RA will be discussed.

3.1.1 DAS28

A commonly used scoring system to evaluate RA patient disease activity, is the Disease Activity Score using 28 joint counts (DAS28) (9, 10, 17, 20-23). Recommended by the EULAR, this scoring system helps defining clinical remission and guiding treatment decisions (21-26). DAS28 is a composite score including acute-phase response markers (mostly Erythrocyte Sedimentation Rate (ESR) and CRP), Swollen Joint Counts (SJC), Tender Joint Counts (TJC) and a Visual Analogue Scale (VAS) indicating a patient's self-reported disease activity (26). One way to evaluate the impact of obesity on RA is to study if and how obesity affects the DAS28 score. Different longitudinal studies have reported a significant association between BMI and DAS28. A commonly used method is dividing RA-patients in different groups based on BMI, a method also used in the report of Ajeganova et al. (20). This study reports an independent statistically significant negative impact of increased BMI on DAS28 (20). Analysis of 1333 RA-patients with a mean follow-up of 9.8 years reported a mean DAS28-score of 2.7±1.3 in normal weight patients while mean DAS28 in obese patients 3.0±1.2 was ($p=0.002$) (20). Another study, by George et al. (27), focused on severely obese women. Adjusted for CRP-levels, DAS28-levels were 0.15-0.29 points higher compared to normal weight women (27). Complementary herewith, other studies found that the DAS28 decrease in response to treatment was less outspoken in overweight and obese patients. Consequently, obesity at the start of treatment is linked with a decreased chance of remission after treatment (28, 29). In line, two research teams agree on a lowered chance of just below 50% of achieving low disease activity in obese patients after 2-3 years (23, 29). However, Nikiphorou et al. (29) did add that after 5 years this difference was gone (figure 3) (29). Sandberg et al. (22) report even a much faster working impact on disease remission in case of obesity: overweight and obese

patients already undergo a 33% lower chance of achieving low disease activity after 3 months (22). Different reports have confirmed an association exists between higher BMI and higher DAS28 (30-33). For example, BMI groups were strongly associated with DAS28 in a French (p=0.002) and Moroccan (p=0.006) population (31, 32). In accord, an international cross-sectional study across 25 countries showed an increase in DAS28-score between normal weight RA-patients and overweight and obese patients of respectively 0.18 and 0.26 points (33). BMI as a categorical variable was independently associated with higher mean DAS28, however, when BMI was used as a continuous variable, a significant positive association between BMI and DAS28 was identified among men but not women. Jawaheer et al. (33) were among the first to indicate in a multicenter study enrolling over 5000 patients from 25 countries a difference in impact of obesity on DAS28 between genders (33). However, it should be noted that this difference was small and not reaching significance, possibly influenced by the number of women being four times as high as the number of men included in this study (33). Baker et al. (34) performed their research on a population of RA-patients who were MTX and biological therapy naive. At baseline, no association with body mass was found (p=0.5 for DAS28-ESR, p=0.9 for DAS28-CRP) (34).

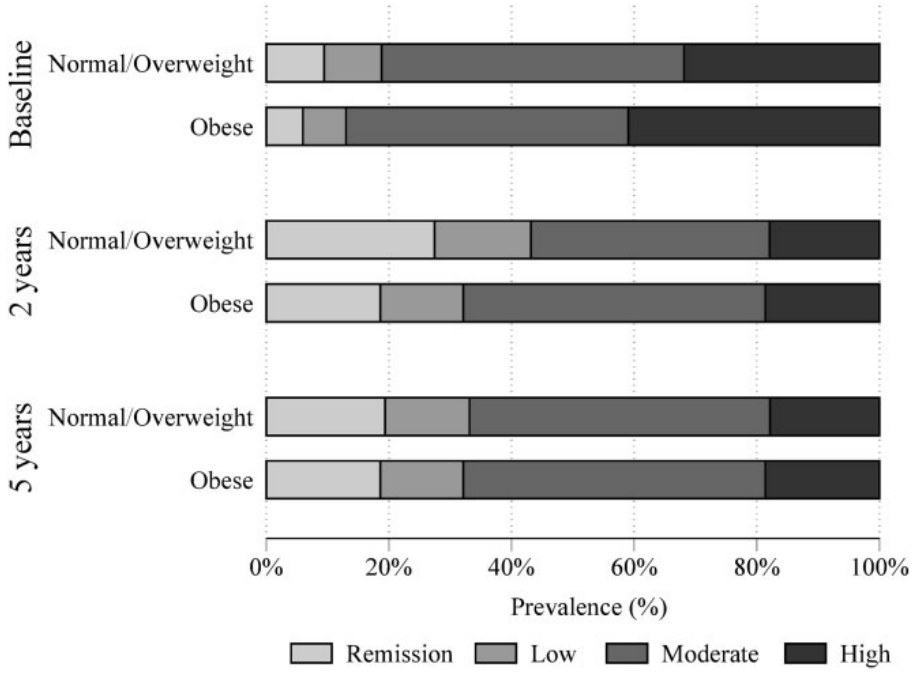

Figure 3: prevalence of DAS28-categories at baseline, 2 years and 5 years of follow-up categorized by BMI-status at baseline. Illustrating the impact of baseline obesity status influencing DAS28 with less remission and low disease activity in the obese population after 2 years, but with no difference between 2 and 5 years of disease (29).

BMI is the most popular way to define obesity. Waist circumference has only in recent years increased in popularity as a tool to assess obesity (20, 35). Hence, while definitions of different BMI-categories are generally agreed upon, cut-off points in defining central obesity by waist circumference display much more variation. For instance, Ajeganova et al. (20) use ≥94cm in men and ≥80cm in women as cut-off points, while Uutela et al. (35) use ≥102cm and ≥88cm as cut-off points. In (20), Waist circumference per 10 cm was not significantly associated with DAS28 ($p=0.4$), while it was associated with Health Assessment Questionnaire (HAQ) ($p<0.001$), a tool discussed extensively in 3.1.2. In contrary, central obesity was associated both with DAS28 ($p=0.019$) and HAQ ($p=0.027$). In (35), waist circumference was associated with DAS28-ESR ($p=0.011$). While most reports find a positive association between obesity and DAS28, Rydell et al. (36) report no association. However, questions can be raised considering this study due to the relative small sample size (n=233), the non-longitudinal evaluation and the lack of reporting of corresponding data (36). Limitations can be almost found in every study and differences in study design and methods make it difficult to compare studies. To illustrate this, an overview on the above mentioned results and studies is given in attachment 1.

Different authors have looked into the different components within the DAS-28 scoring system and compared the impact of BMI on separate components. The different parameters herein can be classified in objective parameters (CRP, ESR and SJC) and subjective parameters (TJC and Patient Global Assessment) (22, 29). Which components of the scoring system are most associated with obesity is described below.

3.1.1.1 Objective parameters

Different reports have drawn different conclusions about the association between BMI and CRP/ESR. In a retrospective longitudinal study with 260 early RA-patients under treatment, over 24 months, Levitsky et al. (28) associated ESR and CRP positively with BMI (28). An association between ESR and BMI was also considered by Ayhan et al. (30), however, those results were non-significant ($p=0.087$) (30). In one study, initiation of DMARD therapy led to a less pronounced decrease in DAS28 after 2 and 5 years in obese patients, solely due to a significant difference in ESR between obese (mean ESR after 2 years: 19.5 mm/h) and non-obese patients (mean ESR after 2 years: 14.3 mm/h) ($p=0.001$ after 2 years and $p=0.028$ after 5 years) (29). Other studies however did not retrieve associations between the acute phase reactants and BMI (20, 27, 33, 35-37). A cross-sectional study compared the median CRP-levels between low-normal weight, overweight and RA patients, which were respectively 7 mg/L (IQR=[3;23 mg/L]), 12 mg/L (IQR=[4;23 mg/L]) and 10 mg/L (IQR=[4;24 mg/L]), confirming lack of association between BMI and CRP-levels ($p=0,39$) (37). Besides BMI and CRP/ESR, the SJC is the third objective parameter used in the DAS28-score. No significant

relationship has been found between SJC and BMI (21, 27-29, 34, 36-40). Only one study found an association between both, but only in a male population and not in females (33).

3.1.1.2 Subjective parameters

Upon investigating the subjective components of DAS28, no consensus is found for the relationship between TJC and obesity. Several studies report there is no relationship between TJC and obesity (27, 29, 34, 36), with a few exceptions being the studies by Heimans et al. (41) with 508 early RA-patients under treatment and Schulman et al. (23) with 982 early RA-patients under treatment. In a one-year follow-up, a difference of 1.4 (95% CI [0.6;2.2]) was found, with worse scores for obese patients (41). This result was largely confirmed by a study showing that overweight associated with reduced rates of improvement in TJC over a 3-year follow-up period (23). Patient global assessment, commonly evaluated by VAS, is another subjective parameter used in DAS28. Although less studied than other components, most studies do report a significant association between higher BMI and lower scores on the VAS (20, 28, 31). A prospective study analyzed DAS28 both in their individual components as well in the subdivision of objective and subjective parameters. While none of the individual parameters associated significantly with BMI, there was a clear difference when including all objective parameters as one and all subjective parameters as one. In comparison to normal weight patients, the odds for a significant decrease over the median during follow-up in subjective parameters were lower, but in objective parameters were similar. This result leads to the suggestion of BMI having higher impact on the subjective parameters of DAS28 than on the objective parameters (22). However, we should note that this assumption is not shared by other authors, e.g. Nikiphorou et al. (29), stating that ESR is the main driver of higher DAS28 in obese patients indicating direct effects on inflammatory mechanisms (29).

3.1.2 HAQ

Besides DAS28, a widely used tool during follow-up of RA-patients is the Health Assessment Questionnaire (HAQ), aimed at measuring quality of life focusing on physical disability (20, 42). In the study of Ajeganova et al. (20), higher HAQ was already at the patient inclusion stage significantly linked with higher BMI categories (p=0.006). This association was only more strengthened at the time of the survey, when RA-patients were followed for an average of 9.8 years (p<0.001). By measuring waist circumference per 10 cm instead of BMI, this association remained strong (p<0.001). Adjusted odds ratio's between HAQ and BMI ≥ 30 kg/m^2 were 1.49 (95% CI [1.17;1.89], p=0.001) and between HAQ and BMI ≥ 28 kg/m^2 were 1,33 (95% CI [1.09;1.63], p=0.004). Based on these results, Ajeganova et al. (20) report a significant association between obesity and negative impact on functional ability (20). Baker et al. (40) defined worsening of disability as in increase of >0.2 in HAQ score. In the FORWARD-cohort, not only were adjusted HAQ-scores higher at enrollment in severely obese patients (β=0.17,

p<0.001), these patients were also at a greater risk of progressive disability compared to less obese patients (hazard ratio (HR)=1.25, p<0.001). These associations were also found in the Veteran Affairs Rheumatoid Arthritis (VARA) registry, a prospective observational multicenter American RA registry including veterans with RA for more than 15 years, using the Multidimensional HAQ (MD-HAQ). Importantly, in the latter study results were confirmed even after adjusting for e.g. comorbidities and disease duration, and additionally for CRP-levels and SJC in the VARA-cohort. The association between obesity and worse HAQ score being significant even after adjustment for possible confounders like comorbidities and inflammatory factors shows that the impact of obesity on HAQ in RA-patients cannot be (solely) explained by other comorbidities or more severe inflammatory environment in the patient (40). This counters the explanation Baker et al. (34) had given in an earlier performed study by him, when the higher risk of having an HAQ-score >1 for overweight patients (odds ratio (OR)=1.87, 95% CI [1.03;3.42]) and obese patients (OR=2.44, 95% CI [1.22;4.86], p=0.01) in comparison to normal weight patients was contributed to the influence of comorbidities for which results were not adjusted. Another retrospective cohort found a significant higher HAQ after 2 and 5 years of disease in obese patients in comparison to normal weight/overweight patients, even after adjustment for possible confounders (p<0.001) (29). Albeit most studies report similar results, namely a strong association between higher HAQ and higher BMI (31, 42), a recent cross-sectional study failed to find any association between BMI categories and HAQ-score (p=0.229) (43).

3.1.3 Radiographic progression

When assessing the impact of obesity on the progression of RA, evaluating the role of obesity on the radiographic progression of the disease is crucial. BMI increases have shown to increase synovitis and tenosynovitis in the generally healthy population (37). One of the most frequently used tools to analyze radiographic progression and assessing radiographic images is the Sharp-van der Heijde score (vdHS), a score comprising an erosion score and a joint-space narrowing score, evaluated by radiologists based on X-rays of hands and/or feet, with higher scores showing more structural damage (32, 34, 36, 44-46). Different prospective cohort studies, with study populations of 152 and 374 RA-patients, have found an inverse relationship between BMI and vdHS-scores in RA-patients: higher BMI is associated with lower vdHS-scores (45, 46). Joo et al. (46) reported an independent inverse association between BMI and severe radiographic progression with an odds ratio of 0.88 (95% CI [0.80;0.97], p=0.008). HAQ, anti-CCP antibody positive, baseline vdHS and 6-month cumulative ESR were independently associated with severe radiographic progression, however, all those associations were positive correlations in contrary to BMI. When looking more specifically at different types of radiographic damage, higher BMI was associated with less joint space

narrowing (while anti-CCP was not), but BMI was not associated with erosion progression (while anti-CCP was). 6-month ESR, baseline vdHS and HAQ were associated with both types of radiographic damage (46). Conversely, one retrospective study (34) did report a negative association between BMI and erosion score (Spearman's ρ=-0.14, p<0.01), however less strong compared to BMI association with joint-space narrowing score (Spearman's ρ=-0.22, p<0.0001) and total vdHS score (Spearman's ρ=-0.18, p=0.0001) (figure 4). Similar associations can be seen when calculating the risk in patients with elevated BMI (>25 kg/m²) of having an elevated erosion score (OR=0.41, 95% CI [0.25;0.66], p<0.001) and joint-space narrowing score (OR=0.3, 95% CI [0.17;0.52], p<0.001) (34). A cross-sectional study showed likewise neither a significant difference between BMI groups and erosion score, nor association between BMI groups and total vdHS score; however, they did find a significant positive association between higher BMI and more joint narrowing (p=0.016) (31), as was later also confirmed by Joo et al. (46). The above findings prompted the suggestion obesity might perhaps lead to a non-erosive phenotype of RA, however, when putting this to the test in a retrospective study, no association was found between BMI and radiographic progression after adjustment for known predictors of radiographic progression (28). In an attempt to clarify the difference in results in the effect of BMI on joint narrowing, differences in analyzing methods and follow-up duration can offer an explanation.

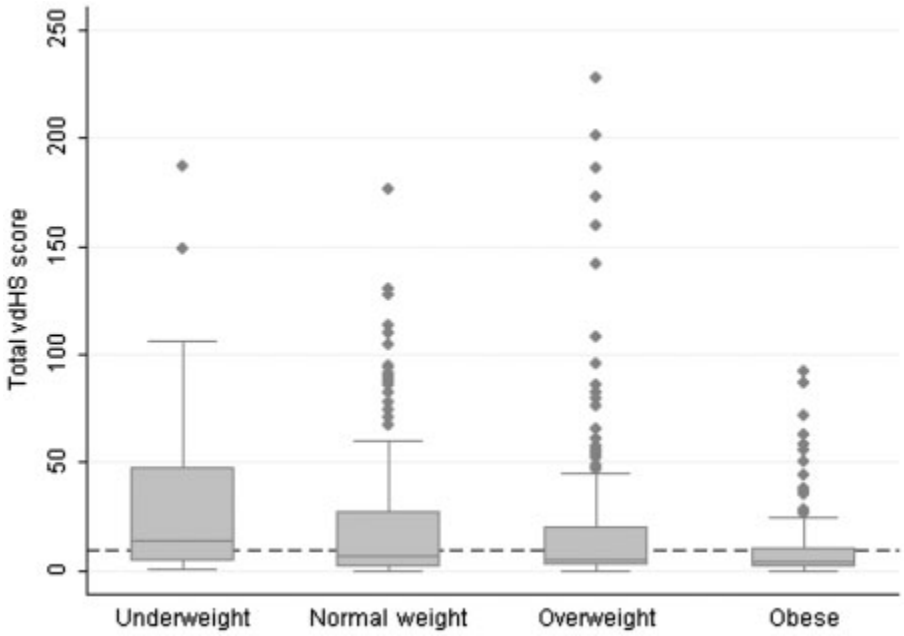

Figure 4: box plot of the distribution of total vdHS scores by BMI category. Illustrating the inverse association between BMI and radiographic damage assessed by vdHS. The dashed line represents the definition of 'elevated in vdHS' (vdHS > 10) in this study (34).

Instead of vdHS and radiographs, other studies used MRI to evaluate radiographic damage. Comparing obese patients to normal weight patients, Baker et al. (44) found less radiographic progression on X-ray after 1 year of follow-up (p=0.002) and less MRI erosion score progression over 2 years of follow-up (p=0.02) in the obese patient group. Furthermore, a greater BMI category was associated with a lower risk of having higher edema score (bone marrow edema is associated with erosive progression) at baseline, independent of other disease parameters or synovitis measured by MRI (no relationship was measured between BMI and synovitis, p=0.09) (44). This may point out a different phenotype of RA between patients with low BMI, namely one with more osteitis and erosion for the same intensity of synovial inflammation, and patients with high BMI, namely a less bone-destructive inflammatory disease (37, 44, 47). Mangnus et al. (37) reported similar observations: a higher BMI was associated with lower MRI inflammation scores adjusted for CRP and ACPA (β = 0.96, p = 0.003), a phenomenon not seen in other arthritic diseases, and while a higher BMI was associated with lower synovitis (β=0.98, p=0.047) and lower bone marrow edema (β =0.95, p = 0.002), no association was found with tenosynovitis (β = 0.98, p =0.21) (37).

While another cross-sectional study of Ayhan et al. (30) shares the assumption of high BMI being linked to lower vdHS (r=-0.158, p<0.01), it should be noted that other cross-sectional studies found different results. One study reported only a borderline association between higher BMI and lower radiographic damage (OR=0.98, p=0.051) (38). Another study used the Larsen score to score radiographic damage, having a similar score system to vdHS by evaluating joint damage based on radiographs of hands and/or feet. This report did not find an association with abdominal obesity (p=0.53) (35). Yet another study reported even a positive correlation between BMI and vdHS (ρ=0.297, p<0.001) (32). Although Baker et al. (34) found an association between higher BMI and less radiographic joint damage, they failed to corroborate the association between baseline BMI and radiographic progression in a one year follow-up (34).

When searching for explanations of opposite conclusions, it is important to analyze other factors besides BMI that can be linked with radiographic progression. Even so, it remains difficult to compare different methods using other statistical analyses to evaluate the impact of different parameters on radiographic progression. A higher ESR is considered to be a factor influencing radiographic progression (33, 34). A prospective observational study reported HAQ as an independent predictor for radiographic progression, but other studies have not analyzed this association (34). There is consensus that the impact of obesity on radiographic progression stays similar after adjusting for CCP-status (29, 34, 36-38, 44). However, one study reports CCP as an independently predictor for radiographic progression, another study reported CCP as independently associated with a greater bone edema score on MRI at

baseline, but not with greater progression over time (44, 46). RF finally did not impact the reported association between BMI and radiographic progression (36). The link between adipokines and radiographic progression will be discussed in section 3.2.1.

3.1.4 Remission

Different cohort studies have examined the impact of obesity on RA disease remission. All studies have identified obesity (BMI) as an independent factor associated with decreased remission (20, 24, 28, 48). Obesity is considered to be the strongest independent predictor for non-remission after 2 years of treatment following the guidelines (OR=5.2, 95% CI [1.8-15.2]), but significantly less remissions in obese patients have also been reported after 6 and 12 months of disease (28, 49). Compared to normal weight patients, overweight patients have on average 25% less chance to achieve sustained remission (HR=0.75, 95% CI [0.58-0.98]), obese class 1 patients 43% less chance (HR=0.57, 95% CI [0.41-0.81]) and obese class 2-3 patients 53% less chance (HR=0.47, 95% CI [0.31-0.71]). Not only higher BMI but also waist circumference at baseline is independently associated with a decrease in chance of remission (20). Other factors identified being independently associated with less remission include being older, being female, being rheumatoid factor positive, smoking and higher HAQ at baseline (24, 28).

3.2 Molecular mechanisms

Studies on the impact of obesity on the progression of rheumatoid arthritis have increasingly gained attention. Besides evaluating this interaction by clinical statistical measurements, many reports have searched for explanations for the associations described in the results section above. A wide variety of study designs and methods have been used in search of molecular mechanisms driving these results: these methods include mouse models, immunoassays, observational studies, etc. I will discuss different pathophysiological mechanisms proposed to explain the associations between obesity and the progression of rheumatoid arthritis on which the most consensus was achieved and to clarify possible contradictory findings.

One of the few reports studying obese RA-patients versus normal-weight RA-patients at a molecular level, is by Alivernini et al. (49). Following immunohistochemical analysis of synovial tissue of the knee in RA-patients resected by ultrasound guided biopsy, obese RA-patients showed a significantly higher number of resident synovial inflammatory cells such as sublining $CD68^+$-, $CD20^+$-, and $CD21^+$-cells. In line with this enhanced synovial inflammation, they reached significantly less remission after 6 and 12 months, regardless to the precise synovitis pattern at disease onset, suggesting the impact of obesity on remission. Remarkably, even after reaching remission, immunohistochemical differences in synovial tissue are still observed between obese patients and non-obese patients, with more sublining $CD68^+$-, lining $CD20^+$-,

lining $CD3^+$- and sublining $CD3^+$-cells in obese patients. These findings also fit the observed delayed remission profile in obese mice (see further below). In the same study, gene expression profiles of different proteins in synovial tissue of RA patients showed that obesity matched a pro-inflammatory state (49).

Results from different mouse models will next be presented to evaluate whether results from human patients can be reproduced in mice. One well-known mouse model is the collagen-induced arthritis (CIA) model, reached by immunizing mice with collagen type 2. The most frequently used mice strains for CIA are DBA/1 and C57BL/6. One group of CIA mice fed a normal diet (often referred to as CIA-lean) compared to another group of mice receiving a high-fat diet (often referred to as CIA-obese or CIA-Ob) represents the most commonly used study design. Comparing between both groups onset of RA-development, RA-severity and inflammatory parameters is crucial to understand the overall impact of obesity. The arthritis score assesses the severity of arthritis in CIA by a scale from 0 ('no edema or swelling') to 4 for each limb ('edema and erythema from the ankle to the entire leg'). Exacerbation of the inflammatory burden can be observed histologically as enhanced infiltration of cells into the joint, synovial pannus formation and increased articular destruction (50-60).

Despite the seemingly straightforward model set-up, different results have been reported. Some reports concluded that obesity accelerates development, aggravates severity and exacerbates inflammatory burden, when comparing CIA-Ob with CIA-lean mice (51, 53, 54). Other reports failed to corroborate the histological impact of obesity on CIA, or found that the difference in arthritis scores between both groups did not reach significance (55, 56, 61). Consensus was reached however on the conclusion that obesity does link to a significant increase in inflammation in CIA-mice (51, 53-56, 61). Consistent herewith, fat-free mice lacking white and brown adipose tissue were protected from mounting the effector phase of RA in a serum transfer K/BxN model of inflammatory arthritis, while fat transplantation reversed this effect, proving the important role of adipose tissue in RA (62).

Metformin, an anti-diabetic drug used for diabetes type 2, one of the biggest comorbidities seen in obese patients, causes weight reduction in obese patients. Considering metformin showed similar effects in rodents, and following the hypothesis that obesity impacts RA, this drug was expected to likewise decrease the severity of arthritis in obese CIA-mice. Indeed, histological bone erosion, cartilage destruction, joint destruction and pannus formation ameliorated in obese CIA-mice receiving metformin versus control treatment. The inflammatory response was significantly decreased in both obese and non-obese CIA-mice treated with metformin, suggesting an important role of obesity in the pathophysiology of RA (50, 57).

3.2.1 Adipokines

Adipokines, proteins produced by adipose tissue and other cells, may provide in a molecular link between obesity and RA (15, 21, 28, 34, 37, 43-45, 49-51, 53-55, 57, 59, 62-83). Indeed, certain adipokines contribute to a low-grade chronic systemic inflammatory state of the body in obese patients and have modulatory effects on bone, cartilage, synovial and immune cells (42, 74, 83). A background on adipokines was presented in 1.1.3.4. In this section, focus is placed on results studying associations between adipokines and disease parameters in obese RA patients, and underlying mechanisms hereof.

3.2.1.1 Adiponectin

3.2.1.1.1 Statistical findings

BMI and serum adiponectin levels show an inverse relationship (34, 45, 67). However, because the relationship between BMI and serum adiponectin is characterized by an exponential decay, smaller differences in adipokine levels are observed between patients with increasing BMI (43). Levels of adiponectin are higher in RA-patients, both in serum and synovial fluid, independent of their BMI, in comparison to other arthritic diseases (15, 66, 69). A cross-sectional study found adiponectin independently positively associated with DAS28-ESR in a significant manner ($p=0.0258$), while BMI was not independently associated with DAS28-ESR but showed a trend of higher disease activity scores in lower BMI categories (43). Conversely, a prospective cohort study observed that while elevated levels of adiponectin at baseline associated with higher disease activity, there is no association between adiponectin levels and disease activity found during follow-up when patients were treated with RA-medications (84). As mentioned earlier, higher BMI is in some reports linked with lower radiographic damage. If adiponectin plays a vital role in this association, higher adiponectin levels may be associated with more (rapid) radiographic progression. Different cross-sectional and prospective studies have found an independent significant association between adiponectin and radiographic progression, especially erosion and joint narrowing, with higher odds of radiographic progression for patients with higher average adiponectin levels, even when adjusted for TNF-α and IL-6. Moreover, this progression was lost when replacing adiponectin by BMI or other metabolic confounders, suggesting that association between adiponectin and radiographic damage is at least partially independent of inflammatory cytokines and fat-tissue status, and thus cannot be the sole explanation for the inverse relationship between obesity and RA joint damage (45, 65, 67). Giles et al. (45) report the probability of radiographic progression is not lowered in obese patients with lower adiponectin levels. This could be the clinical consequence of the decay in the association between adiponectin and BMI as earlier mentioned, or it may indicate that other adiposity-associated factors like e.g. CCP-positivity and gender influence joint damage (45). Reports finding no association between adiponectin and radiographic

progression are a minority (34). Interestingly, biologicals did not change adiponectin serum levels and use of steroids did not impact the association between adiponectin and radiographic damage; combining these facts with findings that adiponectin can predict radiographic progression, adiponectin can act as a valuable marker of disease in RA-patients (43, 65, 67).

3.2.1.1.2 Pathophysiology

A wide range of effects induced by adiponectin has been described. In (78), wild type mice and adiponectin-deficient mice were compared to evaluate the effect of adiponectin on bone metabolism *in vivo*. Adiponectin-deficient mice had significant increased trabecular thickness, number and bone volume fraction, increased tissue bone volume function and increased volume bone mineral density. On top of that, less osteoclasts, decreased RANKL mRNA and protein levels were observed, while osteoprotegerin (OPG) proteins and OPG mRNA's were significantly higher. A higher ratio of RANKL over OPG indicates dominance of osteoclastogenesis. In adiponectin-deficient mice, the $\frac{\text{C-terminal cross-linked telopeptide of type I collagen (CTX)}}{\text{osteocalcin (OC)}}$-ratio, with CTX acting as bone resorption marker, OC as bone formation marker and thus the ratio CTX/OC as bone resorption marker, was significantly lower compared to wild type mice. Summarized, this study indicates that enhanced adiponectin may negatively affect bone metabolism by over-stimulating osteoclast activation, which is reached by stimulating RANKL-expression and inhibiting OPG-expression in human osteoblasts via the AdipoR1-p38 Mitogen-activated protein kinase (MAPK) pathway (78). However, when inducing arthritis in both wild type mice and adiponectin-knockout mice, similar effects on inflammation and joint damage have been reported, showing that RA-induction can be independent from adiponectin (62).

Adiponectin was further shown to stimulate the production of different cytokines, especially IL-6, and MMPs (matrix pro-degradative enzymes) in RASFs expressing adiponectin receptors (15, 16, 43, 45, 65, 67). It is important to note that also IL-6 associates with radiographic progression, as shown for a period of 4 years (67). RASFs play a crucial role in the destructive process of RA in the joints. A crucial role in the invasive RASF mechanism is reserved for adiponectin, mostly expressed in sites of synovial invasion and in the lining layer; it has chemo-attractive effects on RASFs and promotes migration of this group of cells and lymphocytes *in vitro*, leading to the suggestion of *in vivo* effects of increased infiltration of lymphocytes in the synovium and increased influx of RASFs to sites of inflammation, contributing to the degradation of cartilage. In addition, RASFs have a special phenotype determined by a patient's genetic and epigenetic characteristics, contributing to different effects when stimulated by different isoforms of adiponectin, complexing the pathophysiology (15, 16, 68, 74). Adiponectin-stimulated RASFs express chemokines like MCP-1, which attracts

inflammatory cells to synovium to aggravate inflammation, cytokines like IL-6 and IL-11, and matrix-degrading enzymes especially MMP3 and MMP10 (MMPs known to be involved in degrading cartilage). Adiponectin-stimulated human chondrocytes produce predominantly IL-6 and IL-8, and adiponectin-stimulated human lymphocytes secrete IL-8, IL-6, TNF-α and RANTES (chemokine ligand 5, CCL5) (16).

Taken together, adiponectin may be the key to explain the inverse relationship between BMI and radiographic progression: adiponectin can contribute to articular damage in RA patients, and the more visceral fat, the less adiponectin is expressed by adipocytes, resulting in higher abdominal adiposity as protective effect against radiographically recorded damage in joints (figure 5) (21, 43, 45, 64, 67, 81, 85).

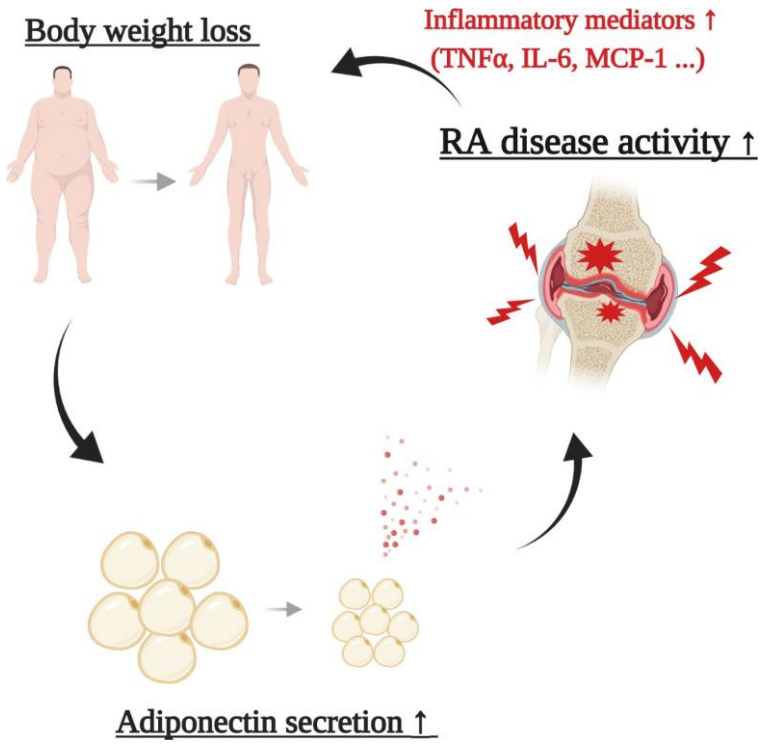

Figure 5: hypothesis about the link between adiponectin and RA. Adiponectin aggravates RA disease activity by production of chemokines/cytokines in joints of RA-patients. These inflammatory mediators may lead to body weight loss, resulting in increased secretion of adiponectin from adipose tissue (43).

Adiponectin is not a homogenous entity however: different isoforms exist, from trimeric adiponectin ('low-molecular weight adiponectin', LMW-adiponectin) to the assembly of 12-18 monomers ('high-molecular weight adiponectin', HMW-adiponectin). Different effects have been described based on the type of isoform (15, 37, 69). Higher-molecular weight adiponectin stimulates more IL-6 production (15). While it is believed all isoforms have similar effects on the pathophysiology of RA, the effects of some isoforms are more destructive than others, with HMW as the driving factor for negative effects in RA, confirmed by the observation of an

independent association between HMW-adiponectin/total adiponectin ratio and DAS28 (15, 37, 69). It is reported that HMW-adiponectin and total adiponectin correlate highly in the healthy population with a wide range of BMI ($r=0.927$, $p<0.001$), but further investigations into the contribution of isoforms in RA-patients are still in progress (65). The concept of different isoforms could explain conflicting results in clinical studies in which adiponectin is measured in its totality and no distinction is made between different isoforms (15, 37, 65, 69). It should also be noted that circulating serum adiponectin levels not always correlate with adiponectin levels in joints (45).

3.2.1.2 Leptin

3.2.1.2.1 Statistical findings

Leptin has been positively associated with BMI, more specifically, its levels are highly associated with adiposity and total fat mass (5, 34, 65, 67, 69, 71, 81, 82). Due to the positive correlation between leptin and BMI in RA patients ($r=0.69$, $p<0.001$), leptin has been used as a proxy for adiposity in RA patients (34). Results are more inconsistent however, especially when evaluating leptin levels in RA-patients when compared to the healthy population or when extrapolating from murine models. Some clinical reports in humans find higher serum leptin levels in RA, even increasing with longer disease duration, while different mice models report decreasing systemic leptin levels by inducing RA (52, 55, 65, 67, 75-77). It should be noted that the reports finding a decrease in systemic leptin levels in CIA-mice have not released full data (52, 55). It has further been suggested that leptin levels are increased in serum but decreased in synovial fluid (77). In the RA-population, differences in leptin levels are also seen between RA-patients with more erosion versus less erosion. RA-patients with more erosion have increased serum leptin, but particularly increased synovial leptin, leading to a higher ratio of synovial/serum leptin in erosive patients which is positively independently associated with radiographic erosion in RA patients (75). It has been reported that leptin is a good surrogate biomarker of disease progression, with a significant correlation between changes in DAS28 (Spearman's correlation $r_s=0.59$, $p=0.008$). In addition, correlation is observed between leptin and IL-17 (Spearman's correlation $r_s=0.49$, $p=0.02$), while no correlation between leptin and IL-6 or TNF-α has been reported (67, 77). This could be due to still increasing leptin levels when other cytokines like TNF-α have reached a plateau, proving a role of leptin in preserving long-term inflammatory state (77). In one report, an association was found with disease activity during-follow up, but only for normal weight patients, suggesting that the effect of leptin is masked by obesity, an assumption earlier made by Baker et al. (34), which can be explained by the effect of adiposity on leptin-endothelial activation (34, 84). Because of conflicting results on the association between DAS28 and leptin, adiponectin is still a considered a better disease biomarker, supported by the many reports proving association between adiponectin and

radiographic damage; this level of support is not the case with leptin (34, 65, 67, 72). Meyer et al. (65) found a relationship between baseline leptin and radiographic progression at 1 year (OR=1.59, 95% CI [1.05;2.41], p=0.03) but it was only associated with erosive damage (OR=1.68, 95% CI [1.1;2.56], p=0.01). However, when radiographic progression was defined as 5 points or more difference in vdHS-score instead of more than 1 point, significance was lost (65). The study by Baker et al. (34) reported a significant association between leptin levels and vdHS, which was not found for adiponectin, but it showed no independent relationship after adjustment for BMI, which was found to be co-linear with leptin levels, and thus is leptin not independently associated with erosion scores (34). Different studies report no significance association between leptin and radiographic damage (65, 67). One of the reasons of these contradictory results could be variation in adjusting for gender; women have higher levels of leptin (69, 75, 81). Nonetheless, higher leptin levels in synovial fluid in osteoarthritis patients was significant associated with more pain (81).

3.2.1.2.2 Pathophysiology

Leptin stimulates RASFs leading to increased production of IL-6 and increased phosphorylation of Signal Transducer and Activator of Transcription 3 (STAT3), both in a concentration-dependent manner. The effect on STAT3 is induced by leptin and not by IL-6, and the production of IL-6 is mediated via the JAK2/STAT3 pathway (76). Leptin stimulates proliferation and survival of T-cells, stimulates the cytokine production by T-cells and polarizes T-helper responses to Th1 (69, 71, 86). Combining with results showing less severe arthritis in leptin-deficient mice, which develop severe obesity due to the loss of anorectic effects of leptin, these results suggest a pro-inflammatory effect of leptin in the pathophysiology of RA (71, 76). However, leptin-knockout mice showed similar joint damage and inflammatory parameters to wild mice when inducing serum-transfer induced inflammatory arthritis, suggesting no crucial role of leptin in the development of RA (62). Considering the higher levels of leptin in obese patients, a positive association between leptin, obesity and disease severity is expected. As earlier mentioned, this relationship is controversial. Furthermore, transgenic mice overexpressing leptin, had less IL-6 expression and no increase in severity of induced arthritis (82).

The above described contradictory findings can be explained by the so-called concept of 'leptin resistance' (28, 66, 71, 82). In a mouse model study, leptin was injected in normal weight and obese mice on two different sites. When leptin was intraperitoneally (peripheral) injected, obese mice showed no weight reduction while normal weight mice did. This difference was lost after injecting leptin intracerebroventricular (central). After injecting leptin in obese arthritic mice, different results were again observed dependent on the site of injection: intracerebroventricular injection did exacerbate peak score of arthritis compared to injection

with control fluid at the same site, while no difference was seen in peak score between intraperitoneal injection of leptin and intraperitoneal injection of control fluid. This suggests that hyperleptinemia (for example in obese patients) can lead to peripheral leptin resistance and explain why hyperleptinemia caused by obesity can reduce inflammation in RA despite the reported pro-inflammatory effects of leptin (71). Having extremely high leptin levels could thus be protective for radiographic damage due to arising peripheral resistance, while modest high levels exhibit their pro-inflammatory and damaging effects on the joints. This fits with the finding that leptin-deficient mice show thymic atrophy and decreased maturation of B- and T-cells; findings that are also reported in obese patients (71, 86). The resistance is peripheral because intracerebroventricular injection resulted in weight loss and worsening of arthritis, whereas these effects are not reached by injecting intraperitoneally. It has been suggested that defective transport of serum leptin through the blood-brain barrier and thus not reaching the hypothalamic arcuate nucleus can help explaining why the resistance is peripheral and why centrally injected leptin still exhibits its pro-inflammatory functions and exacerbates arthritis, probably by central effects on the immune system (71, 86). Leptin resistance could also explain the finding of why an association between RA disease activity and serum leptin levels, consequential to the pro-inflammatory effects of leptin, are solely found in normal weight patients (84).

However, other mechanisms have also been described to explain why obesity links with less radiographic damage but higher leptin levels. One of those mechanisms could be a different inflammatory effect of leptin dependent on its location: central or local in a joint. In erosive RA-patients, synovial leptin concentrations are more in balance with serum leptin levels compared to non-erosive RA-patients, with serum leptin being higher than synovial concentrations, leading to a high synovial/serum leptin ratio. In non-erosive RA patients, not only serum leptin levels are lower compared to serum leptin levels in erosive RA-patients, synovial leptin levels are significantly lower than serum leptin levels of the same patient compared to erosive RA-patients, causing a lower synovial/serum leptin ratio in non-erosive RA-patients. This leads to the suggestion of a protective function of synovial leptin against joint damage by consumption of the adipokine in the joint (75). This could also explain why synovial fluid leptin levels can be reduced in more non-erosive RA-patients (77). Leptin further produces IL-1 receptor antagonist (IL-1RA) which is known to stimulate chondrocyte and fibroblast proliferation hereby and inhibiting joint damage (75, 82). On the other hand, TNF-α can downregulate production of leptin inside the joint, possibly leading to less protective effects induced by leptin in the joint and thus leading to more erosion (75). In hyperleptinemic mice, the production of IL-6 by human macrophage-like-cells *in vitro* was lower than in control mice and leptin-deficient mice were shown to possess macrophages producing more IL-6, suggesting that leptin is necessary

to control the production of IL-6, which is contrary to other what other studies have reported (76, 82). It should be noted that IL-6 production can be reached by different pathways (by stimulation of leptin, TNF-α, …) and this could bias these results (75, 76, 82). So far, the mouse model of transgenic mice overexpressing leptin showing no increase in arthritis score, could either be interpreted as proof of the existence of the concept of leptin resistance, or as proof of no role of serum leptin levels in arthritis progression (82).

3.2.1.3 Resistin

Resistin is a pro-inflammatory adipokine, positively associated with BMI, and is linked with stimulating IL-6 and TNF-α production (2, 4, 5, 45, 53, 67, 69, 72, 73). In RA-patients, it has been reported that resistin levels increase with higher BMI (67). However, in one RA cohort BMI did not correlate significantly with serum resistin (r=-0.045, p=0.83) (73). RA on itself is characterized by higher levels of resistin, in serum and in synovium, in comparison to healthy individuals or osteoarthritis-patients. Because of this, a role of resistin in the pathophysiology of RA is presumed (53, 73). It has already been reported that resistin can stimulate macrophages to produce inflammatory cytokines, that injection of resistin in mice leads to an RA-like disease and that resistin is associated with intra-articular IL-6 and increased white blood cell counts (45, 53, 73). However, both serum and synovial resistin are not associated with ESR, CRP or disease duration, and also the relationship with DAS28 is controversial (53, 73). Conversely, synovial resistin was significantly associated with RF- and ACPA-titers in respectively RF- and ACPA- positive RA-patients. This study also reported no correlation between serum and synovial fluid resistin levels in RA patients (r=0.327, p=0.067) (73). To add to the conflicting results, one study found a significant correlation between resistin synovial levels and serum levels using the radiographic Larsen score (p=0.01 and p=0.02 respectively), yet, another study found no association between resistin levels and vdhS (67, 73). These controversial results suggest that plasma levels of resistin do not always correlate with common markers of RA disease, and that rather synovial fluid resistin levels should be used as prognostic tool to evaluate patients at risk off severe RA disease and less as tool to follow-up disease activity. Resistin can after all be influenced by therapy with DMARDs (73).

3.2.1.4 Visfatin

Visfatin, also known as pre-B-colony enhancing factor (PBEF) or Nicotinamide phosphoribosyltransferase (NAMPT), is an adipokine produced mainly by visceral adipose tissue, exerting its pro-inflammatory effects via the insulin signaling pathway (65, 68, 83). Levels of serum and synovial visfatin are higher in RA-patients compared to healthy individuals, probably mediated by an increase of IL-6, a cytokine significantly associated with visfatin. In RA patients, most studies found no association with BMI, but others did (67, 68, 83). Similar contradictory results have been found in evaluating the association with DAS28

(65, 68, 83). However, methods between studies differed substantially, and when looking at a prospective cohort with early RA-patients, DAS28 was correlated with baseline visfatin levels (r=0.383, p=0.015). After 3 months of follow-up, a similar correlation was found (r=0.338, p=0.018) and decreased visfatin levels after 3 months correlated with and predicted a decrease in DAS28 after 12 months (r=0.354, p=0.027). An association between visfatin and radiographic progression over 4 years has been reported, but seemed entirely dependent on anti-CCP antibodies (67). Because visfatin is involved in B-cell development, this may explain its relationship with anti-CCP antibodies, however, both positive and negative associations between visfatin and anti-CCP antibodies have been reported, adding to confusion (67, 68, 83). The influence of biological therapy on visfatin levels may explain these inconsistent results (68).

In a CIA-mouse model, visfatin was increased locally in the paws, not in serum. APO866 is a small molecule acting as competitive inhibitor of visfatin. By giving APO866 before disease onset, CIA-mice were protected against arthritis, showing ameliorated clinical and histologic findings (reduced synovial immune cell infiltration) and improved radiologic joint damage. Even giving APO866 in the established disease caused dose-dependent improvement of clinical scores, swelling, histologic scores and radiographic damage (significant reductions of cartilage erosion). APO866-administration resulted in down-regulation of chemokines and MMPs, demonstrated by a decrease in IL-1β mRNA, $CD3^+$-T-cells, $LY6G^+$-neutrophils and RANKL mRNA by administration in early RA stage, and a decrease in MMP3, CCL2 and RANKL by administration in established arthritic disease. These results suggest an important role of visfatin in the negative impact of obesity on RA (59). On top of that, visfatin has proven to stimulate in vitro MMP3, CCL2 and IL-8 production by fibroblasts. Visfatin is capable of recruiting leukocytes to the synovium and stimulates them to exercise destructive effects on bone and cartilage. Levels of visfatin appear to be correlated with inflammation, suggesting that infiltrating cells (leukocytes and RASFs) at inflammatory sites secrete visfatin. Effective treatment results in decreased disease activity which is related with a decrease of immune cells in affected synovium, and thus a decrease of visfatin may reflect improvement of disease (59, 68).

3.2.1.5 Chemerin

Chemerin is an adipokine predominantly released by adipocytes, exhibiting both pro-inflammatory and anti-inflammatory effects (74, 79, 83). Its functions are mediated by binding to its receptor Chemokine like receptor 1 (CMKLR1, also known as Chemerin Receptor 23), which is expressed by chondrocytes, macrophages, dendritic cells and NK-cells (74, 80, 83). Levels of chemerin are increased in obese patients and in RA-patients (70, 74, 79, 80). However, it has been reported that in RA-patients, chemerin levels are inversely correlated

with BMI (γ=-0.26, p<0.05) (79). Chemerin is positively associated with DAS28 and even HAQ (70, 74, 79, 83). Furthermore, correlation with IL-6 and other inflammatory cytokines have been found, also in obese RA patients (70, 74). CMKLR1-positive cells are accumulated in synovial tissue of RA-patients (80).

In the general population, chemerin correlates with BMI, waist circumference, body fat percentage, total lipids and even with different markers of chronic inflammation like IL-6 and CRP (74). While chemerin levels are higher in RA-patients, one study reported chemerin being inversely correlated with BMI in RA-patients, suggesting that elevated chemerin levels in obese RA-patients are rather caused by systemic inflammation than by obesity. Chemerin can so be used as disease marker in RA-patients (79). While chemerin may be more associated with systemic inflammation by RA and not with obesity, chemerin may still be a factor to consider when studying the impact of obesity on worsening RA disease. Losing weight in RA-patients with low-moderate disease activity led to decreased levels of chemerin alongside clinical remission (74). Production of chemerin by RASFs can be stimulated by TNF-α and interferon gamma (IFN-γ). This stimulation leads to the production of pro-chemerin, which is converted to chemerin by proteases produced by neutrophils and mast cells at the inflammatory sites. Chemerin binds on different cells expressing its receptor, e.g. macrophages and dendritic cells, activating and chemoattracting them to sites of inflammation, where it further stimulates RASFs leading to an extensive range of effects, with the involvement of CCL2, MMP3, chondrocytes and synovial fibroblasts, finally resulting in bone and cartilage damage (74, 80, 83).

While different reports contradict each other about whether or not chemerin levels are significantly higher in synovial fluid in RA compared with osteoarthritis, activity of chemerin in RA may be higher, leading to more proliferation of synovial fibroblasts (74, 80). Chemerin also stimulates Toll like receptor 4-expression by RASFs leading to osteoclastogenic effects by inducing RANKL (74).

3.2.1.6 Adipsin

Adipsin or Complement Factor D is expressed by adipose tissue and correlates positively with BMI (62, 67). Due to the sometimes unclear role of other adipokines, adipsin has come in sight to understand the impact of obesity on worsening RA (62). One of the crucial pathways in the pathophysiology of joint damage in RA is the activation of both classical and alternative pathway of the complement system. The alternative pathway is the central pathway in inducing arthritis in mice and is considered to be crucial in humans as well. Activation of the complement system leads to neutrophil accumulation into arthritic joints. The alternative pathway can be activated in joints by local components produced by fibroblast-like cells and adipose tissue. Adipsin is a component of the complement system and was shown to regulate neutrophils.

While adipose tissue dominantly releases pro-factor D, it is cleaved into mature factor D by Mannan-binding lectin serine protease 1/3 (MASP-1/3), which is produced by fibroblast-like cells. Therefore, adipsin can link adipose tissue, complement system and RA with each other and can so be considered as one of the components in the complex pathophysiology of obesity and RA. In contrast to results using adiponectin- and leptin-knockout mice, adipsin-deficient mice were consistently shown to be protective against the induction of RA, establishing an important role of adipsin in the mechanisms of RA in a context of obesity (62, 72).

3.2.2 Other mechanisms

In the next section, an overview will be given of different other specific aspects in the pathophysiology that may play a crucial role in the impact of obesity on the progression of RA.

3.2.2.1 IL-1RA

IL-1 Receptor Antagonist (IL-1RA) is an anti-inflammatory cytokine. It has been described in different studies as one of the elements connecting obesity and RA. IL-1RA is produced by adipose tissue and is increased in obese patients. It is not only correlated with BMI in RA-patients ($r=0.35$, $p=0.01$), it is also directly correlated with DAS28 ($r=0.35$, $p=0.01$). On top of that, higher levels of IL-1RA in RA-patients are associated with a less erosive disease, suggesting that obese patients can be protected against bone damage through higher levels of IL-1RA (49). IL-1RA-secretion by monocytes is also upregulated by leptin, explaining the link between higher leptin levels and less bone destruction (75, 82). These findings suggest a possible role of IL-1RA in linking obesity and less radiographic damage in RA-patients.

3.2.2.2 Th17 and T-reg cells

One of the key cellular factors linking obesity and RA are Th17-cells, T-reg cells and the balance between both (50, 53, 54, 57, 58, 82, 86). Th17-cells produce IL-17, interacting with IL-1β and TNF-α to establish pro-inflammatory effects, including in arthritic joints (57, 86). STAT3 is a crucial transcription factor involved in activation, differentiation and proliferation of Th17-cells (53, 58). Different studies demonstrated IL-17 as a crucial factor in the pathogenesis of CIA, via inducing RANKL to stimulate osteoclastogenesis and bone erosion (53, 58, 86). On the other hand, T-reg cells have anti-inflammatory properties by inhibiting proliferation and function of CD4 T-cells. STAT5 is an essential transcription factor for the differentiation of T-reg cells (57, 58). Despite their different inflammatory effects, Th17-cells and T-reg cells are linked with each other; dependent of the inflammatory milieu experienced by the cells, they can be converted in each other, hence an increase in one cell type often is combined with a decrease in the other cell type (57). Grape seed proanthocyanidin extract (GPSE), a flavonoid possessing anti-inflammatory properties, can have therapeutic effects on obesity and RA associated with obesity. It has shown to ameliorate arthritis in CIA obese mice, with a decrease

in STAT3-activity and Th17-cells and an increase in STAT5-activity and T-reg cells, leading to a reduction of synovial hyperplasia, reduced destruction of articular cartilage and bone and a decrease in infiltration of inflammatory cells into the joint (58).

As described earlier, studies administering metformin in CIA mice found a less severe RA-disease (50, 57). Two different pathways were found in which metformin could ameliorate RA: by inhibiting osteoclastogenesis and by optimizing Th17/T-reg balance. More specifically for the latter, metformin caused in CIA mice a decrease in $CD4^+$-$IL\text{-}17^+$-T-cells and $CD4^+$-$pSTAT3^+$-T-cells, and an increase in $CD4^+$-$pSTAT5^+$-T-cells and $CD4^+/CD25^+$-$Foxp3^+$-T-reg cells. It is believed that metformin's primary effect is reduction of pSTAT3, leading to an increase in STAT5 phosphorylation, and thus inhibiting differentiation of Th17 cells and increasing simultaneously T-reg differentiation, leading to a less pro-inflammatory state. 5' adenosine monophosphate-activated protein kinase (AMPK), which inhibits mammalian target of rapamycin (mTOR) and hence also its downstream molecules e.g. STAT3, is crucial in regulating the Th17/T-reg balance. Metformin can activate AMPK pathways and thus have anti-inflammatory effects in obese CIA mice (50, 57). In a CIA-mice study comparing obese to non-obese CIA-mice, similar levels of IL-17-secreting cells were found in the CIA joints, but the expression of IL-17 itself was increased in obese CIA mice. On top of that, higher IL-17 mRNA expression was found in the splenocytes of obese CIA mice, suggesting that obesity stimulates Th17 T-cell differentiation and IL-17 production in the synovium of joints, causing increased joint inflammation (53). However, above reported differences between obese and non-obese CIA-mice were not found in the established disease (54). In search of mechanisms by which obesity can exert this effect, again the adipokines came in sight. It is hypothesized that Th17-cells may have adipokine receptors on their cell surface and can so be influenced by adipokines (53). Indeed, leptin was shown to suppress T-reg cell proliferation in mice and to stimulate Th17-response, as evidenced by increasing IL-17 levels and Th17-cells in synovium during the progression of CIA, due to an increased STAT3 phosphorylation in a concentration-dependent manner. This is associated with exacerbated bone erosion and cartilage damage (54, 76, 86). The correlation found between leptin and IL-17 levels (Spearman's correlation r_s=0.49, p=0.02) fits these findings (77). Interestingly, both STAT3 and Th17-cells are abundant in adipocytes. STAT3 has proven to be crucial both in adipogenesis as in Th17 differentiation, suggesting a common signaling pathway and gene transcription between obesity and the inflammatory state in RA-patients (58).

3.2.2.3 Macrophages and IL-6

IL-6 is a cytokine already mentioned several times. It is a pro-inflammatory cytokine with pleiotropic functions, including regulating the maturation of lymphocytes, macrophages, chondrocytes, endothelial cells and osteoclasts in RA (67, 80). IL-6 can be produced by

macrophages and stimulates macrophages to activate other types of cells or stimulates pro-inflammatory pathways (56, 61, 82). IL-6 is clearly increased in RA-patients, especially in early stages of the disease, and is associated with joint destruction (49, 50, 67). IL-6 is seen as a marker of inflammation due to its correlations with ESR and CRP. It is also linked with RX-progression over 4 years and erosive disease (49, 67). Furthermore, IL-6 has been described as a growth factor for B-cells, which could lead to more anti-CCP antibody-producing B-cells and so explain why the relationship between IL-6 and radiographic progression over 4 years is partially dependent on the presence of anti-CCP antibodies (67).

When administering MR16-1, an antibody against IL-6-receptor, cyclooxygenase-2 (COX-2) expression by macrophages was inhibited and arthritic scores in mice ameliorated (56). While it has been suggested that IL-6 is increased in obese patients, correlations have not always been found (54, 61, 67). Nevertheless, it has been shown that anti-IL6 receptor has more effect in obese RA-patients compared to normal weight RA-patients. An excess of fat may lead to increased IL-6 release, causing residual synovial inflammation, even with good response on treatment. IL-6 is a key factor in the inflammatory response of macrophages, and obesity was shown to stimulate macrophage infiltration and activation, explaining why blockade of IL-6 could have more effects in obese RA-patients (28, 49, 61). Indeed, in obese mice, more macrophages were counted and they were more sensitive to IL-6, as measured by increased expression of COX-2 (54, 56). The change in sensitivity may be explained by a change of macrophage behavior towards a more pro-inflammatory profile, caused by IL-6. An increase in macrophages in RA synovial tissue has also been linked with a deteriorating disease activity (68). This can explain why inflammation and disease parameters are worse in obese RA-patients (49, 54, 56). Macrophages have different phenotypes dependent on their milieu (54). A complex interaction between myeloid cells in obese joint with inflammatory mediators secreted by adipose tissue underpins the pro-inflammatory state of macrophages (2, 4, 8, 54). Furthermore, blockade of IL-6 inhibits differentiation towards Th17 cells and thus has an anti-inflammatory effect (86). As mentioned earlier, adipokines may also help explain why obesity has this impact on IL-6. Adipokines have proven to stimulate IL-6 production in RASFs (16, 33). Similarly, leptin stimulates IL-6 expression, but hyperleptinemia inhibits this, due to leptin resistance (82).

3.2.2.4 FGF21

Fibroblast growth factor 21 (FGF21) is a hormone secreted by different tissues including adipose tissue and thus identified as adipokine. However, the main source of FGF21 is localized in the liver (50, 60, 87). It has been reported that levels of FGF21 in serum and synovium are significant higher in RA-patients. Hence FGF21 is correlated with BMI in patients with RA, but not with lipid profile or disease activity (87). In obese patients, resistance to FGF21

is induced (50). When administering FGF21 to CIA-mice, arthritis score, hind thickness, cartilage destruction and histological damage ameliorated. Immunohistochemically, FGF21 led to a suppressed increase of TNF-α, IL-1β and IL-6 levels compared to control CIA-mice, whereas IL-10 levels were increased. IL-10 is capable of reducing levels of Th17-cells and thus reducing an inflammatory state in obese RA patients, as mentioned earlier (60). When administering metformin, shown to decrease severity in obese CIA-mice, $FGF21^{+}$-cells in spleen tissue and expression of FGF21 in liver were significantly increased in comparison to control obese mice (50, 57). FGF21 may also suppress nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) p65 nuclear translocation, normally playing a crucial role in joint inflammation. These findings lead to the suggestion that FGF21 plays a role in RA, potentially linking obesity with less radiographic damage, and may become the subject of potential new therapeutic agents for RA (60).

3.2.2.5 Anti-CII IgG

Collagen type 2 (CII) is known to be a pivotal protein in the articular cartilage of joints (60). In CIA mice, collagen type 2 (CII) is used to induce arthritis in mice, causing an increase of antibodies against CII, the anti-CII IgG, secreted by B-cells and identified as crucial factor in the induction of arthritis. To assess the impact of different factors on the severity of arthritis, anti-CII IgG may be used as indicator of disease activity (53, 60). FGF21, APO866, GPSE and metformin are known to decrease anti-CII IgG-levels (57-60). Moreover, also T-cells play a crucial role in the induction of arthritis by activation of CII-specific T-cells. It is suggested that fat cells interact predominantly with T-cells. Anti-CII IgG2a antibodies are produced by Th1-helper cells and levels are increased in obese CIA mice in comparison to lean CIA mice, suggesting in obese patients differentiation of T-cells is in the direction of Th1-cells (53). Indeed, leptin has shown to drive Th1-polarisation (69, 86). Th1-responses are well-associated with pro-inflammatory effects in RA (58, 69).

3.2.2.6 Different impact of obesity on different stages of RA

It should be emphasized that obesity can have different effects on the progression of RA, dependent of the stage of the disease. One of the few reports investigating this subject, was the report of Kim et al. (54). In obese CIA mice, the onset of arthritis was earlier due to neutrophil migration induced by the IL-8/MIP2 pathway. Furthermore, other studies have shown that obese mice have higher incidences of developing arthritis after immunization with CII in comparison to control mice. Conversely, when disease progressed beyond day 30, no differences in joint inflammation between obese and non-obese CIA mice were found. In contrast, BMI can delay remission of RA, with a role of pro-inflammatory differentiation of macrophages (53, 54). In a RA-patient study, similar results were found: higher levels of inflammatory cytokines were seen at disease onset in obese patients, and synovial tissue

showed more inflammation in obese patients in remission (49). These findings suggest that obesity exacerbates disease initiation and influences remission, but does not affect established disease (42, 54, 56). The lack of association between obesity and progression of arthritis could explain the contradictory findings between BMI, disease activity and bone destruction.

4. Discussion

4.1 Additional considerations

Besides a main focus on adipokines, many **other mechanisms** have been put forward in trying to explain the link between obesity and RA in view of the many inconsistencies that remain. Despite the contradictory results of lower radiographic impact in obese RA-patients, they still have greater reported disability (27, 47). Obesity is linked with more **pain**, among others by increasing levels of nerve growth factor (NGF), a mediator of pain, possibly expressed by adipocytes (21). With obesity impacting mostly the subjective parameters of DAS28, lower remission rates and higher disease activity scores in obese RA-patients could be related to the extra contribution of obesity on pain, and/or not reflected by changes in the currently used molecular markers that are proxies for an aggravated inflammatory state in obese RA patients. (21, 27, 47).

It has been suggested that pain has both inflammatory as non-inflammatory components. Pain in obese RA-patients may be more inflammatory-driven in the first 3 months of the disease, while the non-inflammatory components contributing to pain such as central sensitization last longer and have more impact, causing high DAS28-scores with predominantly an increase in subjective parameters to be more a reflection of central sensitization of pain instead of active inflammation (22, 26). This fits with conclusions based on inflammatory characteristics in mice models: obesity impacts RA mainly in the initiation of the disease and in remission stages (22, 42, 54, 56). Some studies have proposed some kind of **reverse causation** by pain: higher disease activity in some RA-patients may lead to less physical activity due to pain and thus cause weight gain and obesity. While this mechanism is objected by different studies, it should be kept in mind that being obese as RA-patient can lead to a vicious circle of less exercise, more weight gain and more active disease (20, 22, 33, 42, 88). In an attempt to explain the paradoxical findings of higher BMI being associated with less radiographic damage in joints, other researchers have sought an explanation by studying the opposite situation of the so-called **rheumatoid cachexia**. This concept states that severe RA with serious damage of the joints may lead to unintentional weight loss, causing low BMI to be associated with more radiographic damage and high BMI with protective effects on radiographic damage. Unfortunately, also here controversies exist and more work is needed (40, 44, 47, 63, 88, 89).

It has also been suggested that obesity leading to **increased mechanical loading on bones** can cause increased bone synthesis and is protective against osteoporosis, age-related bone loss. This could explain less joint damage and in particular less erosion in obese patients. More studies are needed to evaluate this possible explanation (21, 34, 44, 46). This mechanism is more pronounced in less inflammatory arthritic diseases like osteoarthritis. It has been

suggested that obese patients have been misclassified as RA-patient when in fact they had osteoarthritis, due to the low-grade inflammation in obese patients making osteoarthritis more RA-like. This may evoke a wrong impression of less radiographic damage in overweight patients in RA. However, the small percentage of patients being misdiagnosed cannot explain the common finding of this association in a wide range of studies (26, 42). Finally, worse subjective pain in obese patients could lead to overtreatment and so less radiographic damage, but this hypothesis is unproven (21).

One of the important factors to consider, is the **different stages of RA** on which obesity can have an impact. Most reports on human patients fail to differentiate between different stages of the disease and make conclusions based on cross-sectional analyses at one or more points in time during the disease. While the aim of this study was to evaluate the impact of BMI on disease progression, the many cross-sectional studies or studies not evaluating the impact of BMI in time during the disease make it difficult to draw conclusions about disease stages. Fortunately, some studies do consider the possible differences in impact during different stages of the disease. Mice models have shown that the impact of BMI was greatest in the early stages and in remission (42, 54, 56). The few studies on human patients confirm this, although the time intervals and concomitant results between measurements differed significantly (22, 29, 46). Almost all studies have one thing in common: at baseline, which in most studies is in early RA-stages, clear impact of obesity on disease parameters is reported, suggesting that obesity indeed influences disease onset (20, 22, 42, 46). More studies are needed to assess what mechanisms play a role in obesity impacting stages other than the stage of established disease, and to what extent. More prospective studies should be set up to assess causality, an aspect which cannot be concluded from cross-sectional analyses.

Potential **confounders**, not discussed in detail, include differences in RF- and ACPA-levels, ethnic differences, gender, comorbidity, microbiota and diet. In addition, genetic and epigenetic factors may influence an individual's response on adiposity and/or arthritic disease and awaits further exploration (20, 22, 27, 30, 33, 63, 84). It is further necessary to be aware of the possible impact of treatment on the described results. Obesity on itself can cause different therapy strategies: drugs doses are often higher in obese patients, and obesity may call for second-choice drugs when obesity is a contraindication for first-choice drug (28, 29). Anti-rheumatic drugs can also induce weight changes in RA-patients. Glucocorticoids are known for their endocrine effects with weight gain as one of the side effects and thus potentially confounding both BMI and disease parameters. This study had not the aim of evaluating the impact of obesity on the treatment responses of RA-patients, but a further exploration of this subtopic could offer new insights in the pathophysiology of both diseases.

One of the big limitations of most studies is the use of **BMI as tool to define adiposity** and obesity, while it is known to be an inadequate marker not reflecting state of adiposity. As mentioned above, adding WC could help in future as a more precise tool to define abdominal obesity (20, 35). Ideally, more accurate tools should be used like bioelectrical impedance assay, dual X-ray absorptiometry or whole-body magnetic resonance imaging (21, 42). Similar questions can be raised about the tools used to assess damage in joints. Imaging is preferred above physical examination of joints, especially in obese patients with adiposity complicating assessment (37). Most studies used radiographic imaging to evaluate damage, while MRI – albeit subject to limited availability – is a more sensitive tool to detect local inflammatory damage.

4.2 Limitations

In this study, I have tried to analyze the impact of obesity on the progression of RA, with special attention to the molecular mechanisms. It is clear that both obesity and RA cannot be defined by one parameter. Both diseases have complex pathophysiological mechanisms comprising different aspects. It is no surprise that the collection of a wide range of different studies are necessary to give an overview of all possible interactions between obesity and RA and their underlying mechanisms. Although an extensive search strategy was performed on different databases and many articles have been read and analyzed, it cannot be stated that this paper gives a complete overview over all aspects. In addition, as mentioned before, methods of studies differed significantly and results were often contradicting each other, emphasizing the need for more extensive research on this subject. Limitations of this paper also include the lack of systematic review of statistical methods used in studies on humans, and the lack of knowledge and experience in analyzing mice models. However, despite these limitations, I have tried to emphasize the most important findings in the context of the aim of this study and to write in a manner that both health care workers and biomolecular scientists can reflect on it.

4.3 Implications for future research

More studies are needed to better understand the impact of obesity on RA. To make conclusions about causality, prospective studies with control groups (non-obese patients) are needed. In an ideal study design, patients being at risk for RA, of which characteristics have been described by the EULAR, should be identified and followed up (90). If this is not possible, a wide range of patients of early RA should be included in a cohort study. When the diagnosis of RA is confirmed, a wide range of characteristics of the patient and his/her disease should be collected. For evaluating the obesity state of a patient, BMI, WC, waist-to-height ratio and waist-to-hip ratio should be evaluated by a doctor following standard protocols and repeatedly measures at every visit. In addition, whole-body DXA-scan should be used to estimate fat

mass; this could provide useful information about body composition and fat distribution, without the possible harmful doses of radiation as used in CT. Blood samples should be regularly taken as well, to analyze among others ESR, CRP, RF and ACPA, but also adipokine levels should be assessed in blood samples. This can be expanded by the analysis of IL-6, IL-1RA and other cytokines. HAQ and VAS should be self-evaluated by the patient, while physical examination needs to assess DAS28, SJC and TJC. Damage of joints should be assessed by MRI when available; radiographic follow-up with vdHS and/or Larsen-score is inferior but can act as alternative for MRI. Drug use during follow-up with special attention to glucocorticoids and changes in therapy strategy should be monitored closely. Follow-up needs to last many years, including the period after possible remission. Different statistical analyses should be performed to assess different possible associations. This ideal setting is practically difficult to perform; however, if some future studies would apply some of these recommendations, more practical information could be gained.

For biomolecular analyses, different techniques could provide useful information about the pathophysiology. When permission is authorized by an ethical committee, abdominal subcutaneous adipose tissue biopsy using an aspiration could provide useful information about gene expression profile and inflammatory characteristics in adipose tissue. The examination of synovial tissue obtained by arthroscopy may offer new insights, however, the biopsy of synovial tissue in a wide range of patients could raise ethical questions. Otherwise, if patients exhibit swelling of joints due to increased amounts of synovial fluid, this fluid can be sampled with less invasive techniques. On biopsies, different examinations can be performed, with the aim to collect information about among others gene profile and the expression of cytokines. Mice models have proven to ameliorate understanding in underlying molecular mechanisms of progression of RA in obese patients. Different arthritis models can be induced in mice and feeding of different types of diet can lead to the distinction between obese and lean mice, mimicking the difference between normal weight and obese human patients. These mice models help to analyze immunohistochemical parameters without performing invasive techniques on humans. However, ethical questions are applicable as well for mice and results of mice models cannot always just be extrapolated to humans. In the end, clinical implications for patients should be prioritized and research on animals should be devoted to this. These biomolecular analyzes should be coordinated with clinical trials and should be considered to be complementary with studies on humans.

The importance of the knowledge about the impact of obesity on the progression of RA cannot be stressed enough. As mentioned in the introduction, obesity is an increasing world-wide health problem with serious consequences for patients, pharmaceutical industry and society. With its growing prevalence, it can be assumed that the numbers of obese RA-patients will

also increase drastically in the following years. To help these patients to the full, understanding of the complex pathophysiology is needed. A better understanding is necessary for adapting and creating guidelines, treatment strategies and customized care for this specific subpopulation of patients. Insights in biomolecular mechanisms in these patients may lead in the long term to drugs with beneficial effects on obese patients, helping them to achieve similar remission rates as non-obese RA-patients. In addition, further studies help raising awareness of the possible impact of obesity on arthritic diseases. Grasping the controversial relationship between obesity and radiographic damage could be of use also for diseases like osteoarthritis, osteoporosis and other musculoskeletal diseases.

4.4 Conclusion

In conclusion, this literature review had the aim to evaluate the impact of obesity on the progression of RA with special attention to the underlying molecular mechanisms. In view of a large amount of studies and an equally large variation in study design contradicting one another, consistent conclusions are difficult to make. What consistently surfaces is that obesity seems to negatively impact disease activity, self-reported pain and disability. In contrast, obesity may have protective effects on radiographic damage. Adipokines in general further link inflammatory states present in both obese and RA-patients. Other potential mechanisms need further exploration. This dissertation has tried to chart the pathophysiology of the chosen topic with attention to the clinical repercussions attached. Without any doubt, also this paper has its limitations and more insights await further research.

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6. Attachments

6.1 Attachment 1: overview of studies and their relevant results cited in section 3.1.1

Reference nr.	Author	Type of study	Patients recruitment	Characteristics of included patients	n = ?	Used statiscal techniques	Relevant results	Statiscal results
20	*Ajagenova et al.*	Retrospective cross-sectional analysis at disease onset and at time of survey	BARFOT-multicenter, a prospective observational study with early RA-patients	Early RA-patients at disease onset, now mean follow-up 9.8 years	n=1596 at disease onset n=1333 at time of survey	t-test, chi-square test, Kruskal-Wallis test, Mann-Whitney U test	a) Significant difference of mean DAS28 in different BMI-categories b) Non-significant association between WC per 10cm and DAS28 c) Significant association between WC per 10cm and HAQ d) Significant association between central obesity and DAS28 e) Significant association between central obesity and HAQ	a) Mean DAS28 (±SD): * BMI ≤20kg/m²: 3.0 (±1.3) * BMI>20 to <25kg/m²: 2.7 (±1.3) * BMI≥25 to <30kg/m²: 2.8 (±1.2) * BMI≥30kg/m²: 3.0 (±1.2) => p=0.002 b) β=0.01 (95% CI [-0.01;0.03]) => p=0.4 c) β=0.09 (95% CI [0.05;0.11]) => p<0.001 d) β=0.04 (95% CI [0.01;0.08]) => p=0.019 e) β=0.09 (95% CI [0.01;0.18]) => p=0.027
27	*Baker et al.*	Retrospective cross-sectional analysis	BC cohort (n=451) and VARA registry cohort (n=1652) for RA-patients, NHANES-study for controls (n=23040)	RA-patients	n=2103	Linear regression models, multivariable models	Prediction from linear regression model differences in DAS28-CRP score between severe obese women and normal weight women	DAS28-CRP score 0.29 points (BC cohort) or 0.15 points (VARA-cohort) higher in severe obese women compared to normal weight women
28	*Levitsky et al.*	Retrospective cohort study	SWEFOT, a 2-year open-label multicenter randomized clinically early RA trial	Early RA patients received at start of follow-up appropriate treatment	n=154	Mann-Whitney U test, Kruskal-Wallis test, Pearson's chi-squared test, Fisher's exact test, odds ratio's by univariate and multivariate analyses	Multivariate model including obesity, female sex, current smoking, HAQ and age, all at baseline, to identify variables predicting non-remission (DAS28≥2.6) at 24 months	Obesity is strongest independent predictor of non-remission: OR 5.2 (95% CI [1.8;15.2])
29	*Nikiphorou et al.*	Retrospective cohort study	Early RA Study and Early RA Network, two multicenter early RA inception cohorts	Early RA-patients under treatment	n=2701	Longitudinal linear mixed effects regression models	Significant differences between DAS28 in obese patients compared to normal/overweight patients at baseline, after 2 and 5 years	* Adjusted DAS28 after 2 years: mean 3.53 in normal/overweight category, mean 3.85 in obese category => p=0.001 * Adjusted DAS28 after 5 years: mean 3.81 in normal/overweight category, mean 3.85 in obese category => p=0.727
23	*Schulman et al.*	Prospective cohort study	CATCH, a multicenter prospective cohort study of Canadian early RA-patients	Early RA-patients receiving appropriate treatment	n=982	1-way analysis of variance, Kruskal-Wallis, Pearson's chi-square test, Kaplan-Meier, log-rank test,	In a fully adjusted survival analysis, obese patients were less likely to achieve sustained remission compared to patients with a healthy weight	* Obese I patients: on average 43% less likely to achieve sustained remission compared to healthy weight patients, HR=0.57 (95% CI [0.41;0.81]) * Obese II and III patients: on average 53% less likely to achieve sustained remission compared

						hazard ratio's with Cox survival analyses		to healthy weight patients, HR=0.47 (95% CI [0.31;0.71])
22	*Sandberg et al.*	Retrospective cohort study	EIRA, a population-based case-control study	Early RA-patients with most of them receiving DMARDs at inclusion	n=495	Logistic regression, odds ratios	Compared to normal weight patients, overweight and obese patients were 33% less likely to achieve low disease activity at the 3-month visit	Odds ratio = 0.67 (95% CI [0.45;1.00])
30	*Ayhan et al.*	Unclear	TRASD-IP register	RA-patients	n=1038	Cross-sectional analyses, regression, multiple comparisons of ANOVA	Obese patients have higher DAS28-scores	p<0.05
31	*Daien et al.*	Retrospective cohort study	ESPOIR Cohort	Early RA-patients	n=628	Chi-squared test, Kruskal Wallis, repeated measures ANOVA and mixed models	Repeated ANOVA showed association between BMI groups and higher DAS28	p<0.0002
32	*Yacoub et al.*	Cross-sectional study	Patients recruited in consultation or during hospitalization at the Department of Rheumatology of the University Hospital of Rabat-Sale in Morocco	RA-patients with mean disease duration of 9.46 years	n=250	ANOVA, Pearson correlation coefficient, regression models	When BMI is used as categorical variable, being overweight was linked with significantly higher DAS28-scores	p<0.006
33	*Jawaheer et al.*	Retrospective cohort study	QUEST-RA program, with RA-patients recruited from 70 sites in 25 different countries	RA-patients receiving usual care	n=5161	Student's t-test, scatter and box plots, ANOVA, multivariate linear regression	Pair-wise comparison by Tukey adjustment showed higher DAS28-values in obese and overweight patients compared to normal weight patients	Compared to normal weight patients: * Overweight patients: 0.18 higher DAS28-score (95% CI [0.05;0.32]) * Obese patients: 0.26 higher DAS28-score (95% CI [0.09;0.43])
34	*Baker et al.*	Retrospective cohort study	GO-BEFORE trial, a randomized control trial used for comparison between different therapy strategies	RA-patients being methotrexate- and biologic therapy naïve	n=499	ANOVA, Kruskal-Wallis, Spearman's correlations, regression models	At baseline, no association is seen between BMI-category and DAS28	a) mean DAS28-ESR (±SD) * BMI ≤20kg/m²: 6.54 (±1.54) * BMI>20 to <25kg/m²: 6.24 (±1.13) * BMI≥25 to <30kg/m²: 6.23 (±1.18) * BMI≥30kg/m²: 6.28 (±1.15) p=0.5 for DAS28-ESR b) mean DAS28-CRP (±SD) * BMI ≤20kg/m²: 5.81 (±1.11) * BMI>20 to <25kg/m²: 5.70 (±1.08) * BMI≥25 to <30kg/m²: 5.75 (±1.06) * BMI≥30kg/m²: 5.74 (±1.03) => p=0.9 for DAS28-CRP
35	*Uutela et al.*	Cross-sectional study	Patients from the Department of Medicine, Central Hospital of Lapland in Finland	RA-patients under treatment having an appointment with a rheumatologist	n=230	t-test, bootstrapped-type t-test, permutation test, Wilcoxon test, chi-squared test, regression models	Abdominal obesity is associated with DAS28-ESR	Patients with abdominal obesity: mean DAS28-ESR 3.5 (SD 1.3) Patients without abdominal obesity: mean DAS28-ESR 3.0 (SD 1.5) => p=0.11
36	*Rydell et al.*	Retrospective cohort study	Patients from the rheumatology outpatient clinic of Malmö University Hospital	RA-patients under treatment with 12 months or less duration of symptoms	n=233	Logistic regression analyses, multivariate models	Categories of BMI not associated with differences in DAS29	Data not shown

6.2 Attachment 2: list of abbreviations

Abbreviation	Explanation
ACPA	Anti-Citrullinated Peptide Antibody
AMPK	Adenosine Monophosphate-activated Protein Kinase
Anti-CCP	Anti-Cyclic Citrullinated Peptide Antibody
BC	Before Christ
bDMARD	biological Disease-modifying Anti-rheumatic Drug
BMI	Body Mass Index
CCL	Chemokine Ligand
CDAI	Clinical Disease Activity Index
CI	Confidence Interval
CIA	Collagen-induced Arthritis
CIA-Ob	Mouse with collagen-induced arthritis and fed a high-fat diet
CII	Collagen type 2
cm	centimetre
CMKLR	Chemokine Like Receptor 1
COX-2	Cyclooxygenase-2
CRP	C-reactive Protein
csDMARD	conventional synthetic Disease-modifying Anti-rheumatic Drug
CT	Computed Tomography
CTX	C-terminal cross-linked telopeptide of type I collagen
DAS28	Disease Activity Score using 28 joint counts
DAS28-CRP	Disease Activity Score using 28 joint counts with C-reactive Protein
DAS28-ESR	Disease Activity Score using 28 joint counts with Erythrocyte Sedimentation Rate
DEXA	Dual Energy X-ray Absorptiometry
DMARD	Disease-modifying Anti-rheumatic Drug
ESR	Erythrocyte Sedimentation Rate
EULAR	the European League Against Rheumatism
FGF21	Fibroblast Growth Factor 21
GPSE	Grape Seed Proanthocyanidin Extract
HAQ	Health Assessment Questionnaire
HLA	Human Leukocyte Antigen
HMW	High-molecular Weight
HR	Hazard Ratio
h	hour
IFN	Interferon
IL	Interleukin
IL-1RA	Interleukin 1 Receptor Antagonist
iNOS	Inducible Nitric Oxide Synthases
IOTF	International Obesity Task Force
IQR	Interquartile Range
JAK	Janus Kinase
kg	kilogram
L	Liter
LMW	Low-molecular Weight
m^2	square metre
MAPK	Mitogen-activated Protein Kinase
MASP	Mannan-binding lectin serine protease
MCP-1	Monocyte Chemoattractant Protein 1
MD-HAQ	Multidimensional Health Assessment Questionnaire
MeSH	Medical Subject Headings

mg	milligram
MHO	Metabolic Healthy Obese
mm	millimetre
MMP	Matrix Metalloproteinase
MRI	Magnetic Resonance Imaging
mTOR	mammalian Target Of Rapamycin
MTX	Methotrexate
NAMPT	Nicotinamide Phosphoribosyltransferase
NF-κB	Nuclear Factor Kappa-light-chain-enhancer of activated B cells
NGF	Nerve Growth Factor
NK	Natural Killer
NSAID	Non-steroidal Anti-inflammatory Drug
OC	Osteocalcin
OPG	Osteoprotegerin
OR	Odds Ratio
PBEF	Pre-B-colony Enhancing Factor
RA	Rheumatoid Arthritis
RANK	Receptor Activator of Nuclear Factor Kappa-B
RANKL	Receptor Activator of Nuclear Factor Kappa-B Ligand
RASF	Rheumatoid Arthritis Synovial Fibroblasts
RF	Rheumatoid Factor
SDAI	Simplified Disease Activity Index
sDMARD	synthetic Disease-modifying Anti-rheumatic Drug
SJC	Swollen Joint Counts
SOCS3	Suppressor of Cytokine Signaling 3
STAT	Signal Transducer and Activator of Transcription
TGF-β	Transforming Growth Factor Beta
Th1 cells	Type 1 T-cells
Th17 cells	Type 17 T-cells
Th2 cells	Type 2 T-cells
TJC	Tender Joint Counts
TNF-α	Tumor Necrosis Factor Alpha
Treg-cells	Regulatory T-cells
tsDMARD	targeted synthetic Disease-modifying Anti-rheumatic Drug
VARA	Veteran Affairs Rheumatoid Arthritis
VAS	Visual Analogue Scale
vdHS	Sharp-van der Heijde score
WC	Waist Circumference