Nutritional impact on health in patients with Rheumatoid Arthritis

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"Qui se ultro morti offerant facilius reperiuntur quam qui dolorem patienter ferant"

- Julius Caesar, 100-44 BCE

Commentarii de bello Gallico, VII.77

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ABSTRACT

Objective: Rheumatoid Arthritis (RA) is the most common autoimmune rheumatic disease, affecting around 0.5-1% of the population. The aim of this thesis was to study dietary impact on markers of health in patients with RA.

Methods: Data from the randomized controlled crossover trial Anti-inflammatory diet in Rheumatoid Arthritis (ADIRA) is used. The trial compares a Mediterranean-like diet intervention with a typical western diet in patients with RA (n = 47). Additionally, cross-sectional analyses were done on data obtained at screening pooled from the ADIRA-trial and a postprandial meal challenge trial in patients with RA (n = 30).

Results: In the ADIRA-trial, apolipoprotein-B100/A1 ratio was improved, high density bound cholesterol increased and triglycerides decreased in the intervention compared to the control. Proinflammatory chemokines decreased compared to control, as well as erythrocyte sedimentation rate in participants with high compliance and no major medication changes. Body composition improved over time during both the intervention and the control diet periods. Developments in nutritional quality differed between the intervention and control diet periods, indicating a successful implementation of the dietary regimens. There was no relation between habitual nutritional quality and health outcomes in a pooled cross-sectional analysis.

Conclusions: Comparing a Mediterranean-like diet to a typical western diet, dietary intake improved cardiovascular risk profile, and in a per protocol analysis, reduced inflammation. Further studies in more diverse populations are required to determine effects on long-term health outcomes.

Keywords: dietary intervention, rheumatoid arthritis, health

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SAMMANFATTNING PÅ SVENSKA

Bakgrund: Reumatoid artrit (RA) är den vanligaste autoimmuna reumatiska sjukdomen, och påverkar cirka 0.5-1% av befolkningen. Även om farmakologisk behandling förbättrats avsevärt de senaste årtionden, är upprätthållen remission över tid inte vanligt. Patienter med RA efterfrågar ofta kostråd, men det saknas i dagsläget evidens för någon specifik nutritionsbehandling vid RA.

Metoder: Den randomiserade och kontrollerade crossover-studien, Antiinflammatorisk Diet vid Reumatoid Artrit (ADIRA) ligger till grund för avhandlingen. Studien utvärderar effekten av en medelhavskost-liknande intervention gentemot en typisk västerländsk kost på patienter med RA (n = 47). I en tvärsnittsanalys med screeningdata från ADIRA samt en postprandiell måltidsstudie (n = 30) studeras dessutom samband mellan kostkvalitet och hälsoutfall.

Resultat: I ADIRA-studien förbättrades apolipoprotein-B100/A1-kvoten, högdensitet lipoprotein-bundet kolesterol ökade och triglycerider minskade av intervention jämfört med kontroll. Proinflammatoriska kemokiner minskade av intervention jämfört mot kontroll, och likaså sänkan minskade hos patienter med hög följsamhet och utan större läkemedelsändringar. Kroppssammansättningen förbättrades oberoende av kostbehandling. Utveckling i kostkvalitet skiljde sig däremot åt mellan intervention- och kontrollkost, vilket tyder på en framgångsrik implementering av respektive kost. I den poolade tvärsnittsanalysen sågs inga signifikanta samband mellan habituell kostkvalitet och hälsoutfall.

Slutsatser: En medelhavskost-liknande intervention förbättrade riskprofil för hjärt-kärlsjukdom och minskade inflammation i en per-protokoll-analys jämfört med en västerländsk kontrollkost. Ytterligare studier krävs i en bredare patientpopulation och under längre tid för att utvärdera långsiktiga hälsoeffekter.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. **Hulander E**, Bärebring L, Turesson Wadell A, Gjertsson I, Calder PC, Winkvist A, Lindqvist HM.

Diet intervention improves cardiovascular profile in patients with rheumatoid arthritis: results from the randomized controlled cross-over trial ADIRA.

Nutr J. 2021 Jan 23;20(1):9. doi: 10.1186/s12937-021-00663-y.

II. **Hulander E**, Bärebring L, Turesson Wadell A, Gjertsson I, Calder PC, Winkvist A, Lindqvist HM.

Proposed Anti-Inflammatory Diet Reduces Inflammation in Compliant, Weight-Stable Patients with Rheumatoid Arthritis in a Randomized Controlled Crossover Trial.

J Nutr. 2021 Dec 3;151(12):3856-3864. doi: 10.1093/jn/nxab313.

III. **Hulander E**, Lindqvist HM, Wadell AT, Gjertsson I, Winkvist A, Bärebring L.

Improvements in Body Composition after a Proposed Anti-Inflammatory Diet Are Modified by Employment Status in Weight-Stable Patients with Rheumatoid Arthritis, a Randomized Controlled Crossover Trial.

Nutrients. 2022 Mar 2;14(5):1058. doi: 10.3390/nu14051058.

IV. **Hulander E**, Lindqvist HM, Turesson Wadell A, Gjertsson I, Winkvist A, Bärebring L.

Associations between nutritional quality of habitual diet, concurrent health characteristics and response to a dietary intervention in patients with rheumatoid arthritis

Manuscript.

CONTENT

ABBREVIATIONS

ACR	American College of Rheumatology
ADIRA	Anti-inflammatory diet in rheumatoid arthritis
anti-CCP	Anti-cyclic citrullinated peptide
APO-A1	Apolipoprotein-A1
APO-B	Apoliprotein-B
APO-B100	Apoliprotein-B100
APO-B48	Apolipoprotein-B48
BCAA	Branched chain amino acids
bDMARD	Biological disease modifying anti-rheumatic drugs
BIS	Bioelectrical impedance spectroscopy
BMI	Body mass index
CETP	Cholesterol ester transfer protein
CRP	C-reactive protein
csDMARD	conventional synthetic disease modifying anti-rheumatic drugs
CVD	Cardiovascular disease
CXCL	C-X-C motif chemokine ligand
DAS28	28-joints disease activity score erythrocyte sedimentation rate
DMARD	Disease modifying anti-rheumatic drugs
DNA	deoxyribonucleic acid
DXA	Dual energy X-ray
ESR	Erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
FAACT	Functional assessment of anorexia/cachexia therapy
FFM	Fat free mass
FFMI	Fat free mass index
FM	Fat mass
FMI	Fat mass index
GH	Global health
HAQ	Health assessment questionnaire disability index
HbA1c	Glycated hemoglobin
HDL	High density lipoprotein
HDL-C	High density lipoprotein-bound cholesterol

IDL	Intermediate density lipoprotein
IGF-1	Insulin-like growth factor-1
IL	Interleukin
LDL	Low density lipoprotein
LDL-C	Low density lipoprotein-bound cholesterol
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated b-cells
NMR	1H Nuclear Magnetic Resonance
Non-HDL-C	Non high density lipoprotein-bound cholesterol
NRF	Nutrient rich foods index
PBMC	Peripheral blood mononuclear cells
PIRA	Postprandial inflammation in rheumatoid arthritis
PREDIMED	Prevención con dieta mediterránea
RA	Rheumatoid arthritis
RBC	Red blood cells
RDI	Recommended daily intake
RF	Rheumatoid factor
SJC	Swollen joint count
SRQ	Swedish Rheumatology Quality Register
TG	Triglycerides
TJC	Tender joint count
TNF-α	Tumor necrosis factor alpha
VAS	Visual analog scale

1 RHEUMATOID ARTHRITIS

1.1 PREVALENCE

Autoimmune diseases affect approximately 4-5% of the global population, and rheumatoid arthritis (RA) is the most prevalent autoimmune rheumatic disease (20). Around 0.5-1% of the global population is expected to be directly affected by RA (21). Symptoms of RA are typically pain, swelling, and a reduced function in peripheral joints (22). During continued disease activity over time, joint destruction often occurs, further diminishing the functional capacity of afflicted individuals. Genetic risk factors, female sex, smoking and advanced age contribute to the development of RA (22). Recent large scale cohort studies have also linked obesity to a higher risk of developing RA (23-25).

1.2 CLASSIFICATION

Today, diagnosis of RA is based on the 2010 American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) classification criteria (26). In brief, when a person presents with at least one inflamed joint not explained by any other diagnosis, without radiographic results typical for RA, a scoring system is applied. This is based on the number of tender or swollen joints (**Figure 1**) in combination with symptom duration and biochemical markers (**Figure 2**). A score of six or above classifies the disease as RA. The 2010 ACR and EULAR classification has a higher sensitivity, and thus captures more patients, than the previously used 1987 ACR criteria (27).

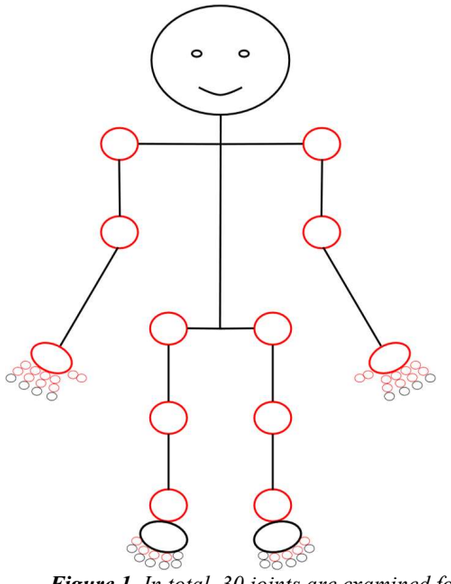

Figure 1. *In total, 30 joints are examined for classification of RA according to 2010 EULAR/ACR criteria, shown here with red circles.*

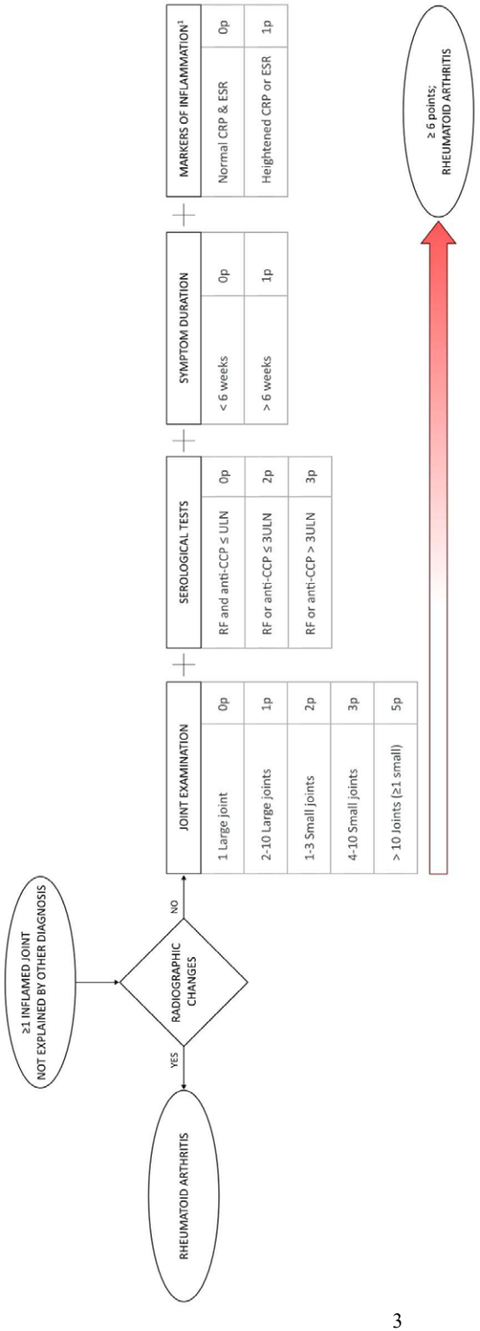

Figure 2. *Classification criteria of RA*

[1]Cut off values for heightened CRP and ESR is determined by local standards.

Abbreviations: anti-CCP; Anti-cyclic citrullinated peptide; CRP, C-reactive protein; ESR, Erythrocyte sedimentation rate; RF, Rheumatoid Factor.

1.3 DISEASE ACTIVITY

In patients with RA, the disease activity often varies over time and in order to assess disease activity, several indexes have been proposed (28, 29). Typically, a combination of the patient's perceived health and an assessment of joint status coupled with biochemical markers of inflammation are used. The most common and accepted measure of disease activity is the 28-joints disease activity score erythrocyte sedimentation rate (DAS28). The DAS28-index is based on an assessment of how many of 28 prespecified joints that are swollen and/or tender, erythrocyte sedimentation rate (ESR)-levels and a patient-reported global health assessment on a visual analog scale (VAS) (range 0-100 mm) (**Figure 3**).

The symptoms of RA has a major impact on quality of life on inflicted individuals, who score lower compared to the general population (as assessed by the 36-item short form health survey (30)) (31). Not only is the general quality of life reduced, disease activity and comorbidities also reduce the life span. The main causes of death appear to be similar between patients with RA and the general population (i.e. cardiovascular-, oncological and respiratory diseases), but disease development is accelerated and, overall, the life expectancy is reduced (32). Managing patients with RA is further costly; the main societal economic burden of RA is related to early retirement and sick leaves (33), this holds true also today, even though pharmacological treatment has improved tremendously during the past decades with the broad application of biological disease modifying drugs (bDMARD) (34).

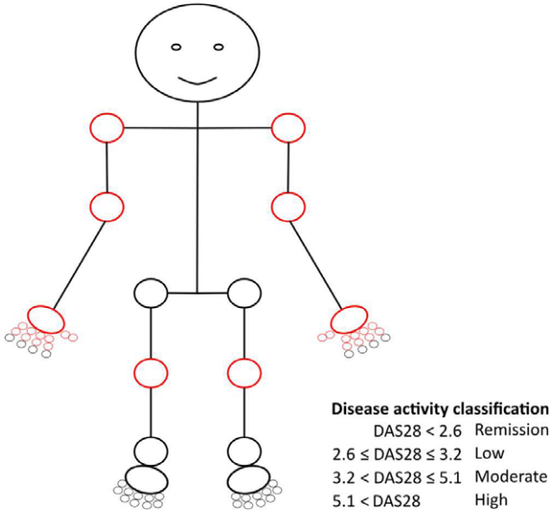

Figure 3. *In total, 28 joints are examined to assess disease activity in patients with RA, shown with red circles here*

1.4 MALNUTRITION AND BODY COMPOSITION

Inadequate nutritional intake, or inadequate dietary quality, in health and disease, may lead to loss of weight and muscle mass, a term referred to as malnutrition. The diagnostic criteria for malnutrition are based either on a low body mass index (BMI), or a combination of weight loss and low fat free mass index (FFMI) (35) (**Table 1**).

Table 1. *Diagnosis of malnutrition in patients identified at risk by nutritional screening*

Either	BMI <18.5 kg/m^2
Or	Weight loss >10%, or 5% within 3 months And either: BMI < 20 kg/m^2 if age < 70 years BMI < 22 kg/m^2 if age > 70 years Or: FFMI < 15 kg/m^2 for females FFMI < 17 kg/m^2 for males

Data from the European society for clinical nutrition and metabolism 2015 consensus statement on the diagnostic criteria for malnutrition (35).
Abbreviations: BMI, Body mass index; FFMI, Fat free mass index.

Dietary intake as well as the metabolism of nutrients can be affected by an individual´s physiology, and many illnesses create a different metabolic environment, a phenomenon commonly referred to as disease related malnutrition. This is perhaps most studied in oncology, where both the disease itself and side effects of treatment can affect dietary intake and metabolism of nutrients. Disease related malnutrition can further be classified into conditions with inflammatory stimuli, or non-inflammatory states. Disease related

malnutrition, with inflammatory stimuli, is commonly classified as cachexia, and is characterized by loss of lean mass (36).

The prevalence of cachexia in patients with RA, commonly referred to as rheumatoid cachexia, is somewhat unclear due to a diversity in diagnostic criteria (**Table 2**). Between reports, the prevalence of rheumatoid cachexia varies between 1% to 54% depending on methodology and patient population (37). Results from a meta-analysis indicate that, if using the most commonly used criteria (FFMI below 10th percentile in combination with a FMI above 25th percentile of a reference population), it appears to have affected about a third of patients with RA (37).

It is not self-evident why cachexia appears in patients with RA. Explanations that have been put forward are related to joint impairment that reduces physical activity and promotes catabolism and fat accumulation, a chronic inflammatory response that induces catabolism, side effects of glucocorticoids as well as an unhealthy diet in general. Further, incidence of RA is increased in the elderly, in particular in middle aged and older females (38). With advanced age, muscle mass and physical functions often decline regardless of underlying disease, a phenomenon referred to as sarcopenia (39). There is thus an overlap between these definitions; many cachectic patients are sarcopenic, but sarcopenic patients are not necessarily cachectic.

In patients with RA, unfavorable body composition (FFMI <25th percentile and a higher than average FMI) is related to higher total serum cholesterol and oxidized low density lipoprotein (LDL) (40), and lower volume of lean mass is related to higher levels of biomarkers of inflammation (41). Data from inpatients in a Swedish hospital has shown that BMI is not a reliable proxy for body fatness in patients with RA (42). Despite a higher or equal BMI compared to the general population, patients with RA presented with lower lean mass, indicating a need to assess body composition directly.

Table 2. *Criteria proposed and used to diagnose rheumatoid cachexia*

Bokhorst et al., (43).	**Engvall et al., (41).**	**Elkan et al., (40).**
weight loss ≥ 5% within 12 months and ≥ 3 of the following: • Low handgrip strength (< 3rd tertile compared to reference) • Low FFMI (< 10th percentile compared to reference) • Low appetite (FFACT questionnaire or VAS scale <50) • Fatigue (VAS >50) • Biochemical markers (ESR >10 mm/1h, CRP >8 mg/L or anemia)	FFMI < 10th percentile and FMI > 25th percentile	FFMI < 10th percentile and FMI > 50th percentile

Abbreviations: CRP, C-reactive protein; ESR, Erythrocyte sedimentation rate; FAACT; Functional assessment of anorexia/cachexia therapy; FFMI, Fat free mass index; FMI, Fat mass index; VAS, Visual analog scale.

1.5 CARDIOVASCULAR DISEASE

1.5.1 BLOOD LIPID TRANSPORTATION

Lipids are transported in the circulation in small particles (diameter about 5 – 1200 nm) with a hydrophilic outside and lipophilic inside called lipoproteins. The lipoproteins are composed of proteins that give structural and functional properties called apolipoproteins as well as phospholipids, cholesterol (in the form of cholesteryl esters and free cholesterol) and triglycerides (TG).

There are different distinct classes of lipoproteins with different functions, a simplified schematic explanation is presented in figure 4. Chylomicrons carry the apolipoprotein-B48 (APO-B48) and transport dietary fat from the intestines to tissues and the liver (mainly TG), the remaining lipids (cholesterol and phospholipids) are transferred to high density lipoprotein (HDL) particles. The chylomicron remnant is then cleared from the circulation by the liver. The main structural protein for HDL-particles, apolipoprotein-A1 (APO-A1), is produced both in the liver and in the intestines. The primary function of HDL particles is to collect and transport lipids back to the liver.

The liver produces TG-rich very low density lipoprotein (VLDL)-particles that primarily transport TG to tissues. Gradually, by unloading lipids, the VLDL-particle is transformed to intermediate density lipoprotein (IDL) and subsequently to low density lipoprotein (LDL). The LDL-particle is taken up either in tissue or in the liver. The VLDL-, IDL- and LDL-particles shares the same basic structural protein, apoliprotein-B100 (APO-B100). VLDL-, IDL-, LDL-particles, and to a lesser extent chylomicrons, are considered to be atherogenic. HDL-particles on the other hand are considered to be atheroprotective.

Some investigations simply quantify apolipoprotein-B (APO-B) as measure of both APO-B48 and APO-B100. In practice, in the fasted state, APO-B concentration primarily reflect the APO-B100 concentration. Elevated blood lipids, measured as either TG, total cholesterol or LDL-bound cholesterol (LDL-C) are considered to be important risk factors for future cardiovascular disease (CVD).

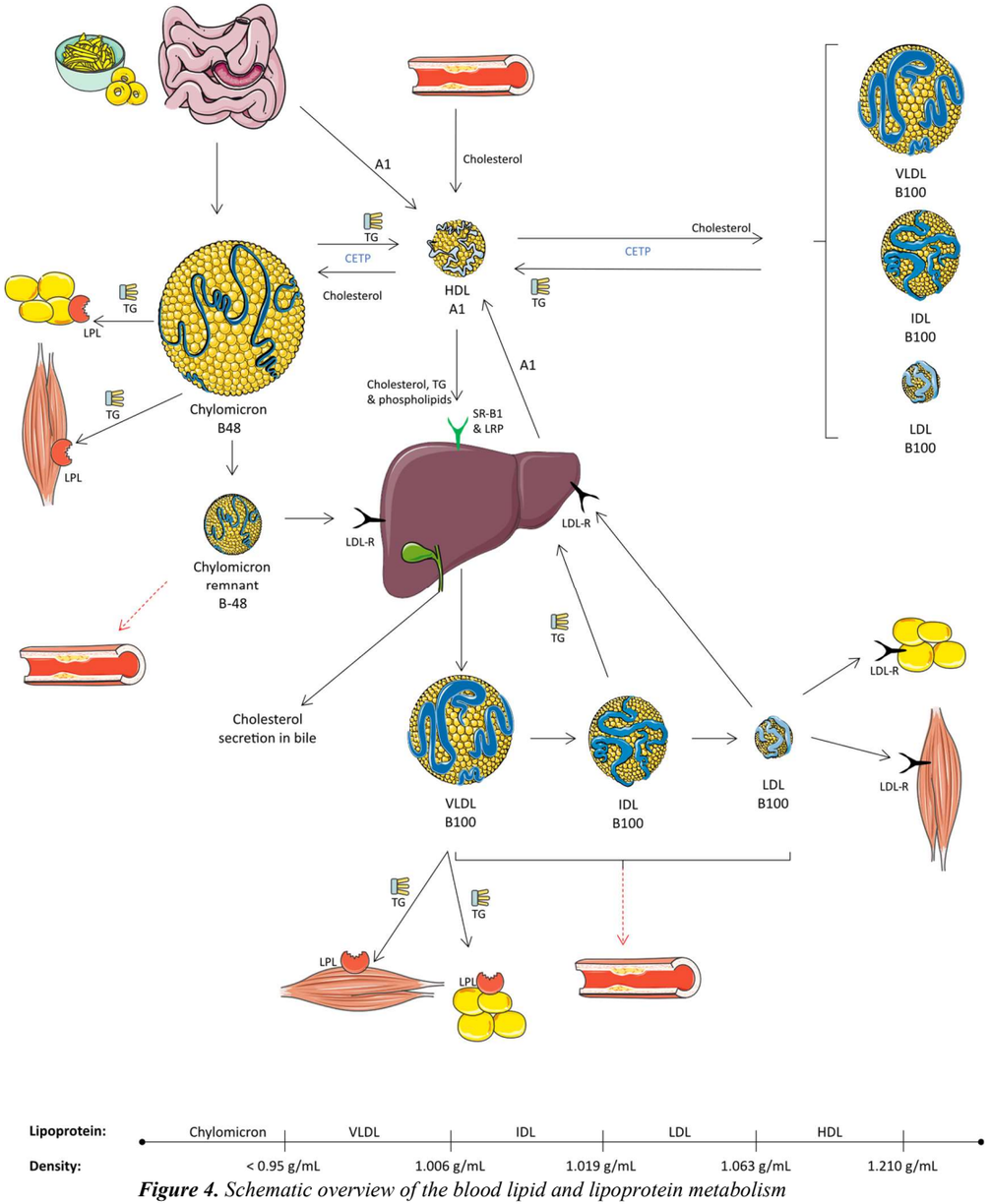

Figure 4. *Schematic overview of the blood lipid and lipoprotein metabolism*

A1, Apolipoprotein A1; B-100, Apolipoprotein B-100; B-48, Apolipoprotein B-48; CETP, Cholesteryl ester transfer protein; HDL, High density lipoprotein; IDL, Intermediate density lipoprotein; LCAT, Lecithin–cholesterol acyltransferase; LDL, low density lipoprotein; LDL-R, Low density lipoprotein receptor; LPL, Lipoprotein lipase; LRP, LDL receptor-related protein; SR-B1, Scavenger receptor, class B type 1; TG, Triglycerides; VLDL, Very low density lipoprotein.

1.5.2 CVD RISK FACTORS

CVD is the most common cause of death in patients with RA (32). The most important modifiable risk factors for atherosclerotic CVD are high circulatory APO-B concentration, elevated blood pressure, smoking and diabetes (44). Chronic inflammation further induces CVD progression, and a relative risk increase of 50% compared to the general population is recommended to assume, when assessing risk of CVD in patients with RA (45). Current recommendations are to assess cardiovascular risk at least once every 5 years in patients with RA, and to measure blood lipid concentrations (45). The main dietary component affecting elevated blood pressure is sodium intake, thus the total salt intake is recommended not to exceed 5 g/day. Additionally, increasing the intake of potassium appears to lower blood pressure (46).

The concentration of LDL particles has long been described as an independent causal risk factor for CVD (47). A reduction in LDL-C has shown to decrease the risk of CVD irrespective of treatment alternative (48). In patients with RA, the lipid metabolism deviates somewhat compared to the general population; inflammation, acute and chronic, lowers the concentration of cholesterol in the circulation (49) (**Figure 5**). As documented by Myasoedova et al. (50), a relative decrease in LDL-C and total cholesterol can be seen even in the years leading up to a diagnosis of RA. Since most cardiovascular risk assessments focus on lipid levels, this can lead to contradictory findings, where patients with a heightened inflammatory state present with low lipid levels. Normalization of inflammation, often achieved by disease modifying anti-rheumatic drugs (DMARD), typically result in an increase in cholesterol levels (Figure 5).

Elevated TG concentration is another established risk factor for future cardiovascular disease. Lowering of TG concentration in blood infers a lower risk for future CVD events, although the effect is not as clear as for lowering LDL-C (51). Both lowering TG and LDL-C are associated with a reduced risk of cardiovascular event, but data suggest that this risk decrease is mediated by APO-B concentrations (52). Recent data further suggest that, when adjusting for APO-B, other lipid parameters no longer significantly predict future

cardiovascular events (53). Thus, when possible, CVD risk assessment benefits by including apolipoprotein concentrations.

While LDL-C has traditionally been the most important marker, recent recommendations point to include non HDL bound cholesterol (non-HDL-C) (essentially a proxy for APO-B carrying lipoproteins) as a potentially superior risk predictor compared to LDL-C (54).

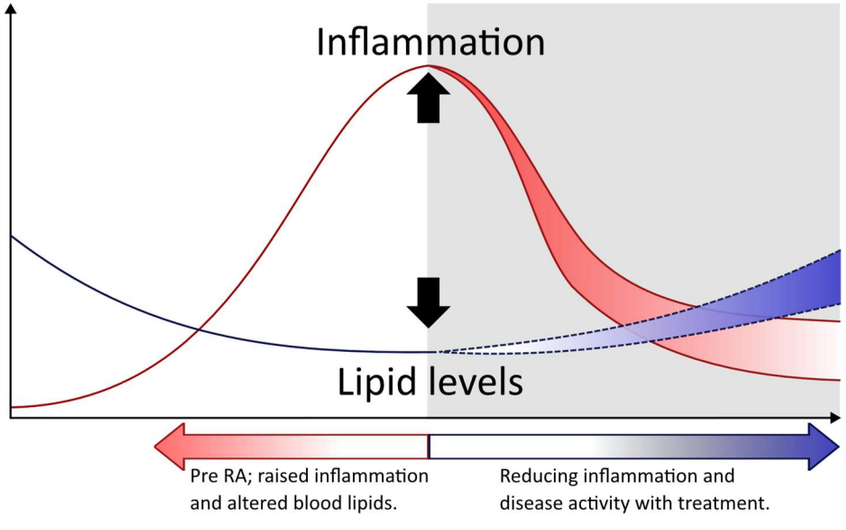

Figure 5. *Blood lipids decrease under concurrent inflammation. Anti-inflammatory treatment that dampen the inflammation, inversely affects the blood lipid levels. Image redrawn and adapted from Choy et al. (49).*

1.6 INFLAMMATORY MARKERS

Patients with RA are characterized by a state of elevated systemic chronic inflammation. Current guidelines recommend quantifying C-reactive protein (CRP) and ESR as clinical markers of inflammation in patients with RA.

CRP is produced and secreted primarily from the liver, and is a pentameric protein composed of five identical monomers. CRP was discovered as a substance (Fraction C) that increased during infection and reacted with the antigenic polysaccharide of streptococcus pneumoniae (55). The half-life of CRP in plasma is about 19 hours, following a tissue damage or infection. CRP production can increase rapidly and typically peaks at 48 hours post injury. CRP is described as having both anti-inflammatory and pro-inflammatory effects; in parts this is hypothesized to depend on the conformation, where pentameric CRP is suggested to dissolve into monomeric form in local tissue, and thereby potentiate the inflammatory response. Most assays, however, do not clearly distinguish between pentameric and monomeric CRP, consequently CRP is commonly interpreted as a general marker of inflammation.

ESR is a long-standing standardized test that together with CRP is the recommended clinical measure to assess acute phase reactants in patients with RA. ESR is an indicator of the relative density between red blood cells (RBC)s and the surrounding plasma, measuring how far the RBCs condense to the bottom of a test tube containing whole blood and an anticoagulant. As recently reviewed (56), RBCs have a negatively charged surface and therefore naturally repel each other. Many plasma proteins have a positive charge, and the increase of plasma proteins alleviate the electrical charge and thereby allow erythrocytes to aggregate and form a rouleaux formation, which allows for a quicker sedimentation. Among plasma proteins, fibrinogen and immunoglobulins have been pointed out as a specifically important factors in this rouleaux formation. The sedimentation rate is further positively correlated to the concentration of RBCs in the blood, and negatively correlated to the plasma transfer protein albumin. Hyperlipidemia is also known to increase the ESR. ESR is therefore appropriately viewed upon as a non-specific marker of inflammation with limited diagnostic value, and with a reference range that varies according to sex and age (**Table 3**). Importantly, deviations from an individual's habitual ESR-value during healthy conditions may confer more information than an individual measurement (57).

Table 3. *Reference range of ESR by age and sex*

	Age <50 years	Age >50 and <70 years
Females[1]	<21 mm/1h	<30 mm/1h
Males	<13 mm/1h	<20 mm/1h

[1]Contraceptives and pregnancy produce values to around 30 mm/1h (57).

The intercellular communication, and thus the response of the immune system, is further regulated by signaling-proteins called cytokines. Cytokines are typically categorized either by their function, or by their structural similarity. Some basic functional classes of cytokines are interferons (antiviral proteins) chemokines (proteins that direct movement of immune cells), anti-inflammatory cytokines (ex. interleukin(IL)-10, transforming growth factor-β), and pro-inflammatory proteins (ex. IL-1, IL-6, tumor necrosis factor α (TNF-α)). However, many cytokines are pleiotropic (i.e., have several different functions) and can act both in an endocrine, autocrine or paracrine manner and be involved in a multitude of processes. (58)

Two cytokines with a central place in the treatment of patients with RA is IL-6 and TNF-α. IL-6 is secreted in a wide range of immune cells as well as endothelial and epithelial cells and is a pleiotropic protein (59), but is generally viewed upon as a proinflammatory molecule.

TNF-α is produced mainly by macrophages and T-cells, but many other cells also produce this molecule, such as neutrophiles, B-cells, NK-cells, monocytes, dendritic cells and also epithelial and endothelial cells (59). TNF-α exist both as a membrane bound protein and in a soluble form in the circulation with a half-life of around 14 minutes (59). TNF-α is generally considered a proinflammatory marker that controls apoptosis and promotes inflammation, often explained by its effect on the transcriptor factor nuclear factor kappa-light-chain-enhancer of activated b-cells (NF-κB) (59). Inhibition of TNF-α, by antibody therapy, is a common and often effective pharmacological treatment in patients with RA. Primarily IL-6, but also TNF-α, induce expression of CRP in liver cells (60).

Chemokines are further a subclass of soluble cytokines that share structural similarities, the primary function of chemokines is to direct to movement of immune cells, who move towards a higher concentration (58). Primary source of chemokines are immune cells (macrophages, dendritic cells, T-

lymphocytes, fibroblasts) as well as endothelial cells and platelets (61). Often, the chemokine receptors are non-specific and can bind to several different individual chemokines (58).

2 TREATMENT OF RA

Pharmacological treatment of patients with RA has improved dramatically during recent decades with the arrival of potent anti-inflammatory bDMARD such as TNF-α and IL-6 inhibitors. Treatment with bDMARDS are often applied in combination with metotrexate which is also overall the most common treatment (62). Patients responding to bDMARDs substantially reduce their cardiovascular risk profile (63). But not all patients are responders, and non-responders can present a residual risk of cardiovascular incidence up to double that of the general population (63). Furthermore, remission over time is a rare occurrence; data suggest that only about one in four patients with RA retains remission over a 12-month period (64). While the potential impact of smoking cessation and physical activity on health outcomes for patients with RA is clear, there is no evidence for any specific dietary treatment (45). An overall healthy diet and lifestyle is recommended.

2.1 DIETARY INTAKE

There is a high prevalence of belief among patients with RA that dietary intake can affect the disease severity (65-68). Dietary changes, instigated without professional medical advice, appear to be common (69). Following a strict and unsupervised diet may however result in nutritional deficiencies, thereby leading to a further diminished nutritional status. There are several indications that diet matters; higher intake of fish (70) and unsaturated fats (71) have been associated to lower disease activity in patients with RA. Likewise, overall diet quality is inversely associated with inflammation in patients with RA (72).

There are several nutrients with proposed immunomodulatory effects. Omega-3 fatty acids is perhaps the most well described nutrient that mediate the immune system (73), the main function is the production of less proinflammatory eicosanoids in competition with omega-6 fatty acids (**figure 6**), thereby facilitating a resolution or alleviation of inflammation. A higher proportion of omega-3 fatty acids in blood has also been correlated to lower concentrations of proinflammatory, and higher concentrations of anti-inflammatory cytokines in the general population (74). In addition to effects on inflammation, omega-3 fatty acids decrease the circulating TG concentrations by increased lipid oxidation and decreased lipid production in the liver (75).

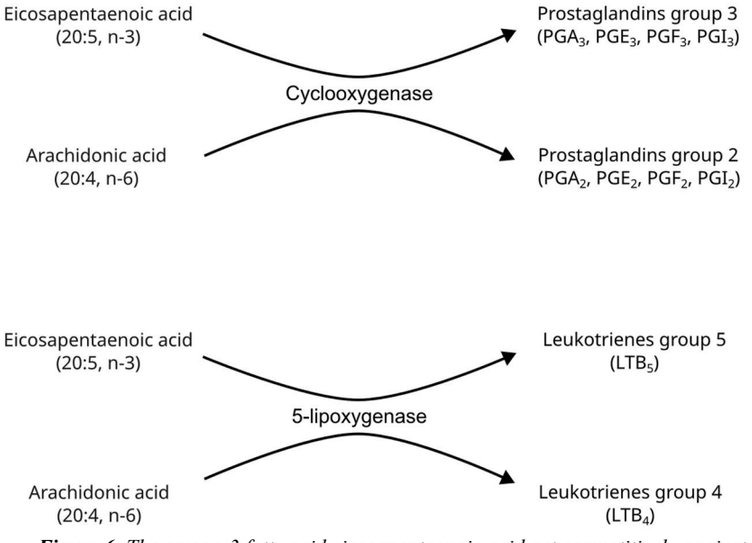

Figure 6. *The omega-3 fatty acid eicosapentaenoic acid act competitively against arachidonic acid and produce less potent proinflammatory molecules. Figure redrawn and adapted from Tosi et al. (76).*

Fiber intake is another dietary component likely to have physiological effects. For example, an adequate fiber intake is indicated to promote satiety, intestinal epithelial integrity, improved glucose metabolism as well as lowering the circulating cholesterol concentration (77).

2.1.1 DIET AND CVD RISK FACTORS IN RA

Aside from an adequate fiber and omega-3 fatty acid intake, several other dietary factors affect blood lipids. The central dietary advice for patients with hyperlipidemia is, except for weight normalization, to improve their dietary fat quality. Several saturated fatty acids are indicated to decrease the LDL-receptor activity whereas several unsaturated fatty acids have the opposite effect, thereby altering the concentration of LDL-C in circulation (78). In patients with RA, one intervention study has seen lowering of cholesterol concentrations in blood after a vegan exclusion diet (5) (presumably combining high fiber intake with improved dietary fat quality). This trial was however troubled by simultaneous weight loss, which naturally affect the lipid transportation in blood (79). Other Mediterranean-like diet interventions have yet failed to lower blood lipid levels in patients with RA (1, 16).

2.1.2 DIET AND INFLAMMATION IN RA

When assessing dietary effects on inflammation, it is rather clear that obesity instigates chronic inflammation, and that weight reduction among the obese has anti-inflammatory effects regardless of diet composition (80), by reducing proinflammatory cytokines, and improving insulin sensitivity.

The whole-diet intervention trials performed on patients with RA have often been of small sample size, and quite often the intervention has both resulted in weight loss and a high dropout rate (**Table 4**). To date, there is only one controlled whole-diet intervention study that has seen improvement in one of the clinically validated biomarkers of inflammation (CRP or ESR), without unadjusted and uneven weight loss between study arms. The study by Sarzi-puttini et al (13) tried a hypoallergenic diet higher in unsaturated fatty acids compared to a control diet. The intervention was devoid of wheat, egg, milk, strawberries, tomato, chocolate, dried fruit, acid fruits as well as crustacean products, and low in red meat. Instead of these foods, the intervention diet was high in hydrolyzed milk, cornbread and corn flour, fresh pineapple and cooked apples. Both diets were designed to be weight-normalizing for the participants, and when analyzed adjusted for BMI-developments, ESR (but not CRP) was reduced by the intervention

2.1.3 DIET AND BODY COMPOSITION IN RA

In terms of dietary intervention on patients with RA aimed at improving body composition, data are rather scarce. One previous trial examined the effects of an amino acid supplement enriched with β-hydroxy-β-methylbutyrate compared to placebo, but saw increased lean mass over time regardless of treatment (81). Another trial assessed creatine supplementation and found that patients with RA, just like patients without RA, increased in lean mass following creatine supplementation, but saw no functional benefit (82). As of today, to the best of our knowledge, there has been no diet intervention assessing effects on body composition in patients with RA.

2.1.4 NEED OF FURTHER STUDIES

As to date, the potential impact of diet on health outcomes in patients with RA is rather unclear, and remains to be determined. The dietary impact on cardiovascular risk profile, inflammation and body composition under controlled, weight stable conditions, remains to be determined. There is a need to assess efficacy of dietary interventions in patients with RA.

Table 4. Previous food-based intervention studies on the biomarkers of inflammation CRP and ESR in patients with RA

Author	Intervention	Design	Length	Weight change	N Included	Dropout (I / C)[1]	CRP	ESR
Barnard et al., 2022 (5)	Vegan exclusion diet with reintroduction vs placebo supplement	Randomized crossover trial	16w		44	27% I/C No info		
Dawczynski C et al., 2009 (6)	Omega-3 enriched dairy products	randomized, double-blind, placebo-controlled crossover study	12w	No info.	45	13% I/C No info	No info.	No info.
Dennisov et al., 1992 (9)	Hypoallergic anti-inflammatory diet vs no diet intervention	non-randomized parallel trial	4w	No info	92	0% / 0%	No info.	No info.
Elkan AC et al., 2008 (10)	Vegan vs balanced non-vegan diet	randomized parallel trial	1 year		66	29% / 21%	No info.	No info.
Hansen GV et al., 1996 (11)	high-protein, fish och vegetarian foods, omega-3, selenium & vitamin A,C & E supplements	randomized parallel trial, single blinded	6 mo	NS.	109	26% I/C No info	No info.	
Holst-Jensen SE et al., 1998 (12)	Peptide based / Elemental diet vs habitual diet	randomized parallel trial, blinded assessor	4 w, (+ 6 mo)[1]		30	13% / 7%		
Kavanagh et al., 1995 (14)	Modified elemental diet & food reintroduction vs habitual diet supplemented with elemental nutrition	randomized parallel trial	24 w	↓ at 4w NS at 24w.	47	46% / 78%		
Kjelsen-Kragh et al., 1991 (15)	Fasting and gluten-free vegan or vegetarian vs "normal"	randomized parallel trial, blinded assessor	13m		53	0% / 0%		
Kremer et al., 1985 (17)	Mufa- & pufa rich diet with marine oil, vs control resembling average population intake	randomized double blind parallel trial	12 w	NS	52	15% / 16%	No info.	
Lindqvist et al., 2018 (19)	1 meal of blue mussel vs chicken/ham/beef, 5 days/week	randomized crossover trial	11 w	NS.	39	41% I/C No info		
McKellar et al., 2007 (1)	Mediterranean diet cooking classes versus brochure on healthy eating	non-randomized parallel trial	6 w, (+ 3 mo)[1]	NS.	130	0% / 0%		

Table 4 continued. *Previous food-based intervention studies on the biomarkers of inflammation CRP and ESR in patients with RA*

Author	Intervention	Design	Length	Weight change	N Included	Dropout (I / C)[1]	CRP	ESR
McKellar et al., 2007 (1)	Mediterranean diet cooking classes versus brochure on healthy eating	non-randomized parallel trial	6 w, (+ 3 mo)[1]	NS.	130	0% / 0%		
Nenonen et al., 1998 (2)	raw food vegan vs habitual diet	randomized parallel trial	3 mo		43	14% / 5%		
Panush RS et al., 1983 (7)	hypoallergenic diet vs active comparator	randomized double blind parallel trial	10 w	No info.	33	21% I/C No info	No info.	
Sarzi-Puttini et al., 2000 (13)	hypoallergenic diet vs active comparator	randomized double blind parallel trial	24 w	NS.	50	12% / 16%		
Skoldstam et al., 2003 (16)	Mediterranean diet vs habitual diet	randomized parallel trial	12w		56	10% / 7%		
van de Laar MA et al., 1992 (18)	Allergen-free enteral nutrition versus enteral nutrition w lactoproteins & azo-dyes	randomized double blind parallel trial	12 w		94	16% /18%		

Green color denotes decreases, gray indicates no significant change. [1]: Follow-up after completed study.

AIM

The aim of this thesis was to explore the relation between dietary intake and biological markers of health in patients with RA.

The specific aim of each paper in this thesis was as follows:

I. To study if dietary intervention can alter cardiovascular risk profile in patients with RA, and if so, what type of characteristics that predispose for response

II. To study effects of dietary intervention on markers of inflammation in patients with RA

III. To study if body composition is altered by a dietary intervention in patients with RA
and what patient characteristics that predispose for treatment response

IV. To explore the role of nutritional quality of habitual diet in relation to health outcomes in patients with RA, and the development in nutritional quality during a diet intervention study

3 METHODS

3.1 DATA SOURCES

This thesis is based on data from two clinical intervention studies performed in patients with RA, one whole diet intervention study and one postprandial meal challenge study.

3.2 THE ANTI-INFLAMMATORY DIET IN RHEUMATOID ARTHRITIS (ADIRA) TRIAL

The ADIRA-trial aimed at determining the efficacy of combining foods with potential anti-inflammatory effects into a proposed anti-inflammatory portfolio diet, compared to a typical western diet (nutritionally similar to the average nutritional intake in Sweden). The ADIRA-trial was approved by the local ethical committee (976-16 and T519-17), registered in ClinicalTrials.gov (NCT02941055), was carried out from February 2017 until May 2018. The main outcome in the ADIRA-trial was a change in DAS28 as a consequence of altered dietary intake.

The outcomes included in the papers forming this thesis are secondary outcomes from the ADIRA-trial; biological markers of inflammation, risk factors for CVD and body composition. Additionally, this thesis has taken advantage of data from screening in the ADIRA-trial, and evaluated markers of inflammation, body composition and DAS28 in cross-sectional analyses.

3.2.1 STUDY DESIGN & PARTICIPANT SELECTION IN THE ADIRA-TRIAL

The ADIRA-trial was designed as a randomized controlled crossover trial (**Figure 7**); sequence was computer-generated and assigned by random at screening. A sample size of 38 was needed for 90% power to detect a change in DAS28 of 0.6 units ($\alpha = 0.05$). To account for dropout, 50 participants were recruited.

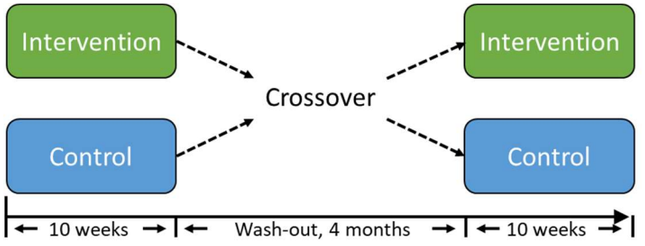

Figure 7. *Median (25p, 75p) duration of intervention diet period were 9.6 (9.3, 9.9) weeks, intervention diet periods 9.6 (9.0, 9.9) weeks, the washout time between dietary treatment was 16.9 (10, 17) weeks.*

Potential participants, with diagnosis of RA listed at Sahlgrenska University Hospital, were identified in the Swedish Rheumatology Quality Register (SRQ) (n = 1091). Those residing on an address where the company mat.se (procured to provide study foods) delivered food were invited to participate (n = 774). In total, 113 (15%) patients responded, and out of those 66 fulfilled pre-screening criteria and were thus called to screening. Inclusion criteria prescreening were age ≥18 years and ≥2 years disease duration. Exclusion criteria at screening were any known life threatening diseases, pregnancy or lactation, allergies to any of the foods in the study, or inability to confirm an apprehension of study instructions. Out of these, 50 patients were invited and randomized by a computer-generated list (allocation ratio 1:1) to take part in the study. Data from 47 patients who completed at least one diet period, and 44 who completed two diet periods were available for analysis from the ADIRA-trial. (**Figure 8**).

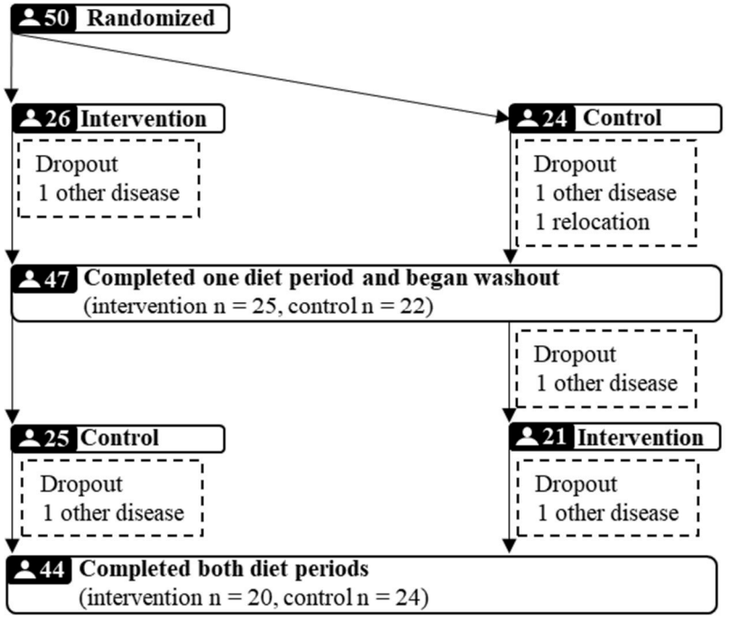

Figure 8. *Flowchart according to the CONSORT recommendation on patients in the ADIRA-trial.*

Participants were interviewed midway into each dietary period, and answered questions on how many of the prescribed meals had been consumed during the past week. For each day a basic compliance score was calculated; consuming all of a meal (breakfast, the main meal, and snacks) gave 100%, some parts of a meal 50%, and nothing of the meals 0%. Every meal was given the same weight, and an average score over the past week was calculated for each participant. A cut-off at > 80% was used to define participants with high compliance.

In general, the self-reported compliance was high, with a median (25p, 75p) of 100% (93%, 100%) during intervention and 100% (87%, 100%) during control among those completing at least one diet period. The proportion of participants with high compliance (i.e., >80%) was 96% during the intervention diet period and 85% during control diet period (**Table 5**).

Table 5. *Participant data material used from the ADIRA-trial*

		Total	With BIS measurements[1]	Without major medication changes[2]	With high compliance[3]
Completed one diet period	Intervention	45	41	38	43
	Control	46	41	38	39
Completed both diet periods		44	40	36	35

[1] Participants with metal implants were excluded from measurements of body composition.
[2] Without new or discontinued DMARD or glucocorticoid treatment during any of the diet periods.
[3] Participants who reported > 80% compliance.
Abbreviations: BIS, Bioelectrical impedance spectroscopy .

Medication, prescribed and self-administered, were recorded by participants at screening, and during the study visits after each diet period. Interval, dose, and time of each substance was compiled. The reported medication usage after each visit was compared to usage at screening to identify changes. All drugs were then categorized by substance class. In statistical analysis, changes in medication were defined as complete cessation or a newly instated drug.

In order not to convolute the dataset too much, changes in timing, dosage or other medications than conventional synthetic (cs)DMARDs, bDMARDs or glucocorticoids were ignored in the statistical analysis. If a participant was started on a new, or completely discontinued any of these drugs during any of the diet periods, all data from that individual was deemed as potentially affected by altered medication. Changes in medication were considered in a sensitivity analysis when assessing cardiovascular risk factors (Paper I) and in the main analysis of biological markers of inflammation (Paper II).

Changes in csDMARD-treatment were noted for: azathioprine, ciklosporin, metotrexate and sulfasalazine. Changes in bDMARD-treatment were seen in: abatacept, certolizumab pegol, etanercept, golimumab, infliximab, rituximab.

Changes in glucocorticoid usage was noted for: betamethasone, prednisolone and unspecified injections.

3.2.2 DIETARY INTERVENTION

The intervention in the ADIRA-trial was facilitated by home-delivery of foods and recipes of easy to prepare meals, along with basic dietary guidance.

During the intervention diet period, participants were asked to limit red meat to ≤ 3 times/week, consume ≥ 5 servings of fruit, berries or vegetables daily. Further, choosing oil and margarine over butter, selecting low-fat dairy products and whole grain products was encouraged. During the control diet period, participants were asked to eat red meat ≥ 5 times/week, consume ≤ 5 servings of fruits, berries or vegetables daily. Further, cooking in butter and the use of whole-fat dairy products was encouraged and use of probiotics was dissuaded.

During both diet periods, participants received a partial food plan covering 1100 kcal/day (**Table 6**), during 5 days per week, allowing for 2 days per week of self-sufficing intake. During the intervention diet period, foods were fatty fish, legumes, whole grain products, nuts, fruits, berries and vegetables, in contrast to the control diet period where the focus was on refined grains, red meat, chicken and protein-rich snacks (protein bars, quark-yoghurt mix or protein pudding) (**Table 7)**.

To avoid potential impact from varying climate conditions and seasonal habits, the intervention and control diet periods were designed to be evenly balanced over the year (**Figure 9**). The intervention periods occurred only slightly more frequent during spring, and consequently the control periods were slightly more frequent during autumn.

Table 6. *Average daily nutritional contents of the supplied foods*

	Intervention	**Control**
Energy (kcal)	1100	1120
Protein (g)	48	65
Fat (g)	45	32
Carbohydrates (g)	114	136
Fiber (g)	20	10
Iron (mg)	9	8
Beta-Carotene (µg)	1768	148
Retinol equivalent	191	133
Vitamin D (µg)	10	1
Vitamin E (mg)	13	6
Riboflavin(mg)	0.9	1.6
Niacin equivalent	23	29
Vitamin B12 (µg)	6	3
Magnesium (mg)	324	161
Potassium (mg)	2474	2137
SFA (g)	9	12
MUFA (g)	15	12
PUFA (g)	17	5
Cholesterol (mg)	69	150
Monosaccharides (g)	32	23
Disaccharides (g)	23	34
EPA (Fatty acid 20:5)(g)	0.7	0.0
DPA (Fatty acid 22:5)(g)	0.4	0.0
DHA (Fatty acid 22:6)(g)	1.3	0.0
Selenium (µg)	45	27
Vitamin K (µg)	85	14
Iodine (µg)	55	108
Starch (g)	17	55
Copper (mg)	0.7	0.4
Antioxidants (mmol)	11	4

Macro- and micronutrients where difference was ≥ 10% between the intervention and the control diet plan. Individual fatty acids are excluded, as well as sodium which was not reliably accounted for. Values are calculated in and exported from DietistNet.

Abbreviations: DHA, Docosahexaenoic acid; DPA, Docosapentaenoic acid; EPA, Eicosapentaenoic acid; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids; SFA, Saturated fatty acids.

Table 7. *Overview of the home-delivered foods in the ADIRA-trial*

	Intervention	**Control**
Breakfast	Probiotic shot, frozen berries or pomegranate and a variation of; ✓ Low fat sour milk and nuts ✓ Fiber enriched oat porridge, skimmed milk, walnuts ✓ Low fat yoghurt, whole grain muesli	✓ Orange juice and white bread with butter and cheese, or ✓ Protein rich yoghurt with cornflakes and orange juice
Hot meal	✓ Fish 2-5 (average 3.8) times /week ✓ Legume based vegetarian food 0-3 (average 1.2) times/week ✓ Whole grain products and vegetables in every meal	✓ Red meat 3-4 (average 3.5) times/week ✓ Chicken 1-2 (average 1.5) times/week
Snack	✓ 2 fruits per day (apple and banana)	✓ A portion of quark-yoghurt mix per day, or ✓ A protein bar, or ✓ a portion of protein pudding

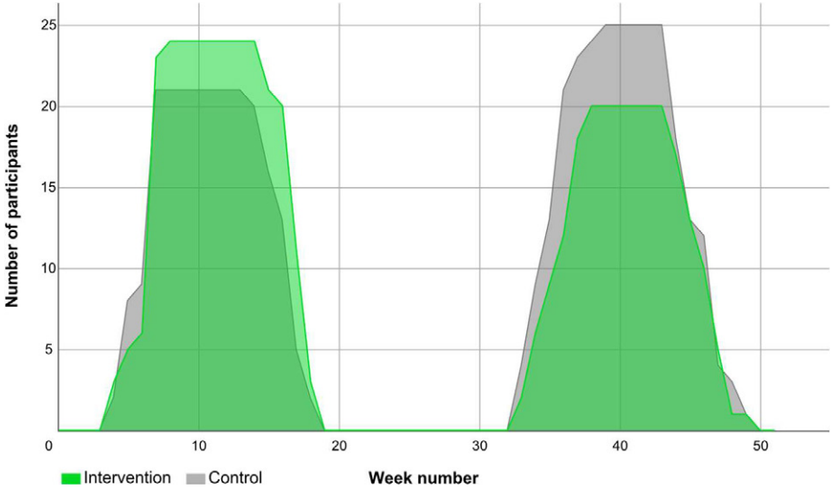

Figure 9. *The number of active patients per week stratified by treatment. Active weeks are defined as the week a participant had the first home delivery of foods until the week that post-period measurements occurred.*

3.2.3 OUTCOMES FROM THE ADIRA-TRIAL INCLUDED IN THIS THESIS

3.2.3.1 PAPER I

Clinical markers of blood lipid transport (total- HDL- and LDL-cholesterol as well as TG-concentration) were analyzed in fresh samples in the fasting state before and after each diet period. The samples were analyzed according to clinical routine at the Sahlgrenska University Hospital, by enzymatic colorimetry using a Cobas 8000 instrument from Roche Diagnostica, Scandinavia AB.

To explore lipid transport particles numbers, APO-B100 and APO-A1 concentration, and cholesterol and TG concentrations in particles, frozen samples were analyzed in a serie by ^{1}H Nuclear Magnetic Resonance (NMR) analysis (83) at the Swedish NMR Centre. Fatty acid composition in plasma was analyzed externally in a serie from frozen samples using gas chromatography (84) at the University of Southampton, United Kingdom. Blood pressure was measured by nurses at the Department of Clinical Rheumatology Research Center before and after each diet period.

3.2.3.2 PAPER II

Clinical biomarkers of inflammation were assessed by routine analysis of CRP and ESR at the Sahlgrenska university Hospital in fresh samples before and after each dietary period. An exploratory analysis of inflammation-related proteins in serum samples was analyzed externally by Olink Proteomics AB, Uppsala, Sweden using the Olink® Target 96 Inflammation panel (85). The analysis of inflammation-related proteins was done on a subset of serum samples where standardized handled serum samples were available (n=26) (**Figure 10**).

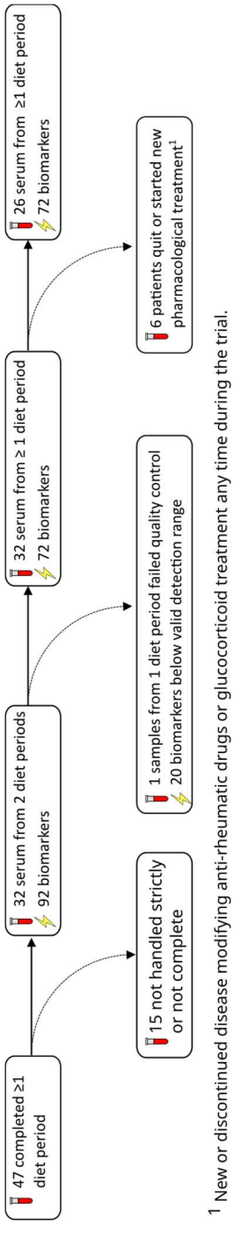

Figure 10. *Serum samples used for quantification of inflammation-related proteins*

3.2.3.3 PAPER III

Body composition was measured by bioelectrical impedance spectroscopy before and after each diet period in the fasted state after 5 minutes in a supine position using ImpediMed SFB7 (ImpediMed, Brisbane, Australia) and disposable electrodes from the Fresenius Kabi Body Composition Monitor product line. Weight was measured in habitual clothing and 1 kg was subtracted to account for clothing.

Metabolites examined for relation to body composition, branched chain amino acids (BCAA) and insulin-like growth factor-1 (IGF-1), were analyzed in a serie from frozen serum samples. BCAA concentration was quantified by NMR analysis, Albumin and IGF-1 were quantified in serum following routine procedures by the clinical laboratory at Sahlgrenska University Hospital.

3.2.3.4 PAPER IV

In addition to outcomes evaluated in paper I, II and III, a nutritional index score based on NRF11.3 (86, 87), as described further below, was calculated and used as an index of nutritional quality in paper IV.

Data was also acquired from screening, where patients filled questionnaires on demographical background, employment status and of habitual physical activity. Waist to hip ratio and weight were measured to the closes 0.5 cm. Participants also disclosed their medication lists.

3.2.4 CONTRIBUTION TO THE ADIRA-TRIAL BY THE PHD-CANDIDATE

The PhD-candidate has collected and analyzed data from the ADIRA-trial, but was not originally involved in conceiving of or developing the study.

3.3 THE POSTPRANDIAL INFLAMMATION IN RHEUMATOID ARTHRITIS (PIRA)

The PIRA-trial is a randomized controlled cross-over trial studying the postprandial effects of intake of meals of different composition (**Figure 11**). The PIRA study was approved by the regional ethical review board in Gothenburg (Dnr 2019-05242) and registered on ClinicalTrials.gov (NCT04247009). Recruitment for PIRA began in January 2020, and clinical study visits began in February 2020, but had to be halted in March 2020 due to the COVID-19 pandemic. The trial was later resumed in August 2021 and completed in November 2021.

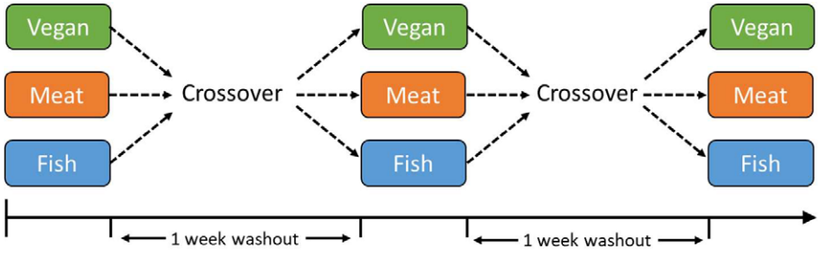

Figure 11. *The randomized controlled cross over trial PIRA*

The primary aim was to evaluate if isocaloric meals composed of different protein and fat sources would affect inflammation and metabolic response differently in patients with RA, and if patients with RA respond differently compared to healthy controls. The main protein source was either minced meat (mix of pork and beef), salmon or a soy protein product (Figure 11). Patients with RA were planned to consume all three meals, and healthy controls only the meal of minced meat.

3.3.1 MEAL COMPOSITION

The meals were developed and carefully calculated and standardized to differ at maximum 1 gram in protein, fat and carbohydrate between meals. In order to best accommodate standardization, burger-based meals were developed. Each meal consisted of two burgers, white bread, salad, cucumber and tomato along with hamburger dressing, calculated energy contents were totaling at 700 kcal/meal. Differences in water and fiber content were ignored. All meals were fried with standardized amount of canola oil and heated to the prespecified internal temperatures of 75˚for minced beef, 52˚for salmon and 70˚for the soy-protein based dish.

Analysis of the chemical composition of the meals was done externally (Eurofins Food & Feed Testing, Sweden) to validate the nutrition content. Differences in amount of fat in the calculated meals versus the analysis were compensated by enriching the hamburger dressing with varying amounts of canola oil. Differences in carbohydrates and protein content was left uncorrected.

3.3.2 RECRUITING PARTICIPANTS WITH RA

Female patients with confirmed diagnosis of RA (ICD-code M05.9 or M05.8) listed at the Sahlgrenska University Hospital was identified in SRQ. Inclusion criteria for this database export was ≥ 2 years disease duration, an age span of 20-70 years and no ongoing treatment with IL-6 inhibition during the past four weeks. Presumptive participants (n=934) were sent letters of invitation, of which 83 responded. Responders were double checked for diagnosis and those breastfeeding or pregnant, or who self-reported obesity (>30 kg/m^2), smokers or with intolerance to any of the trial foods were excluded. Eventually, 42 patients were screened.

3.3.3 SCREENING OF PARTICIPANTS WITH RA

During screening, weight, height, waist to hip ratio, body composition, ESR, CRP, hemoglobin concentration and HbA1c were measured in the non-fasting state, and food records were administered.

Weight and body composition were measured on a void bladder in standardized hospital gown without shoes by Multi-frequency bio-electrical impedance analysis (Tanita MC-180 MA, Tanita, Tokyo, Japan), dual energy X-ray (DXA) technique using Luna Prodigy (Lunar Prodigy, enCORE software 12.30.008, GE Health Care, Madison, WI, USA) and bioelectrical impedance spectroscopy (BIS) using ImpediMed SFB7 (ImpediMed, Brisbane, Australia). Height was measured to the closest 1 cm and waist-hip ratio to the closest 0.5 cm.

CRP, ESR, hemoglobin and glycated hemoglobin (HbA1c) were measured in fresh samples according to the clinical routine at the Sahlgrenska University Hospital. DAS28-ESR was estimated by nurses at the department of clinical rheumatology research center at the Sahlgrenska University Hospital. Participants were also asked to complete 4-day food records. The participants' health status and medication were further checked during interviews and from patient journals, those fulfilling any of the following exclusion criteria were excluded;

- Obesity (> 30 kg/m^2)
- Diagnosis of cancer, inflammatory bowel disease, celiac disease, diabetes
- Allergy or intolerance to any of the foods in the study
- Pregnancy or breastfeeding
- Use of any blood lipid lowering medication, glucocorticoids or IL-6-inhibitor during the past 4 weeks
- No DMARD-changes for the past 3 months
- Smoking
- Hemoglobin < 100 g/L
- HbA1c levels above age-standardized reference

After excluding participants on the above criteria, eligible patients (n=37) were invited to participate in the postprandial meal challenges.

3.3.4 RECRUITING HEALTHY CONTROLS

Healthy controls, defined as not having a rheumatic diagnosis, were advertised for in social media and by word of mouth. Inclusion criteria were female sex and age in the range of 20-70 years. Out of those responding to advertisement, controls were matched to the patients with RA based on age, BMI and physical activity level. The same exclusion criteria as previously described for patients with RA were applied also to controls. No medication or diagnoses were checked for, and joint status was not examined, but otherwise controls went through the same procedure as patients with RA.

3.3.5 POSTPRANDIAL MEAL CHALLENGE FOR PATIENTS WITH RA

Participants came to the study center in the fasted state and were randomly assigned a meal sequence. Spot urine was collected, and blood drawn before the meal. Twenty minutes were allotted for consumption of the meal and additional blood was then drawn after 30, 60, 120, 180 and 300 minutes (**Figure 12**). RBCs were collected only at the first meal challenge, serum, plasma, blood, peripheral blood mononuclear cells (PBMC) and urine samples were extracted during all visits.

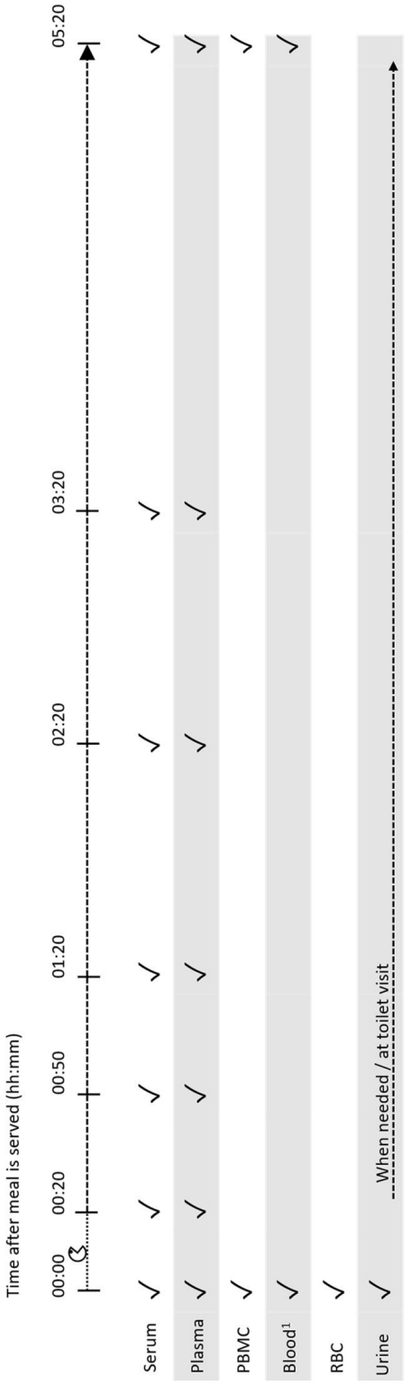

Figure 12. *Times of postprandial sample collection during the meal challenges*

3.3.6 POSTPRANDIAL MEAL CHALLENGES FOR HEALTHY CONTROLS

Healthy controls were only served the meal of minced meat, and in contrast to patients with RA, no PBMC-samples were collected and no whole blood was analyzed, but otherwise the same protocol was used (Figure 12).

3.3.7 OUTCOME MEASURES IN THE PIRA-TRIAL

The main outcome of the PIRA-trial was the postprandial development of IL-6 in serum. Secondary to IL-6, inflammatory and metabolic markers such as CRP, TG, apolipoproteins and gene expression analysis of inflammation-related genes. Serum and urine will also be used for analyses of metabolomics.

3.3.8 OUTCOMES FROM THE PIRA-TRIAL INCLUDED IN THIS THESIS

Due to the COVID-19 pandemic and subsequent trial delays, only the data from screening that were performed in a similar way in the ADIRA-trial, analyzed in a cross-sectional dataset, are included in this thesis; body composition as measured by BIS, CRP, DAS28-ESR, ESR and dietary intake as recorded in 4-day food records.

3.3.9 CONTRIBUTION TO THE PIRA-TRIAL BY THE PHD-CANDIDATE

The PhD-candidate worked on forming the hypothesis, meal development, selection of biomarkers to analyze, the power calculation, ethical approval application, study protocols as well as the recruitment and execution of the PIRA-trial. The trial unfortunately had to be halted due to the pandemic, in the second run, the candidate worked with planning the organization of preparations and meal challenges, re-recruitment of patients with RA, and processing of biological samples.

3.4 COMMON METHODOLOGY IN THE ADIRA- AND PIRA-TRIALS

3.4.1 ASSESSMENT OF NUTRITIONAL QUALITY

Assessment of nutritional quality was performed based on the dietary intake reported in the food records (3-day in ADIRA and 4-day food records in PIRA). A composite score of the intake of some key nutrients, Nutrient Rich Foods Index (NRF)-11.3, previously identified to be of importance to a Swedish population (87), was used. In brief, for each participant, the percentage score of recommended daily intake (RDI) of key nutrients were summed, and the percentage score of negative nutrients were subtracted, leaving a final composite score (**Table 8**). In paper III, this was computed on density values, i.e. adjusting all nutritional intake to an assumed 2000 kcal/day for females and 2500 kcal/day for males. In paper IV, it was calculated both as density and as absolute score, and the absolute score was used for the main analysis.

3.4.2 ASSESSMENT OF DISEASE ACTIVITY

In both studies, joint examinations were performed by the same research nurses at Clinical Rheumatological Research Centre at Sahlgrenska University Hospital.

3.4.3 ASSESSMENT OF BODY COMPOSITION

Assessment of body composition was performed with several methods in the PIRA-trial, and only by BIS in the ADIRA trial. The BIS-measurements were also not done in exactly the same way in the two trials. However, the same machine, with the same type of electrodes and a similar protocol of a 5-15-minute supine position prior to measurement were applied in the ADIRA and PIRA trials.

3.4.4 ASSESSMENT OF PHYSIAL ACTIVITY

Physical activity was assessed as described in paper III in both trials. Based on scales between 1 and 5 on both habitual physical activity and intentional physical exercise, a physical activity index between 1 and 4 was calculated, resembling what was previously tried and validated by Wareham et al. (88).

Table 8. Composite nutritional score according to the NRF11.3

	Score cut-off		Maximum value
Positive scoring nutrients	**Women**	**Men**	
Calcium (mg)	800	800	1
Fibre (g)	25	35	No limit
Folate (µg)	300[1]/400[2]	300	1
Iron (mg)	9[1]/15[2]	9	1
Magnesium (mg)	280	350	1
Potassium (g)	3.1	3.5	1
Protein (E%)[3]	15	15	1.33
Vitamin A (retinol equivalents)	700	900	1
Vitamin C (mg)	75	75	1
Vitamin D (µg)	10	10	1
Vitamin E (mg)	8	10	1
Negative scoring nutrients	Score cut-off		
Added sugars (E%)	10	10	No limit
Saturated fat (E%)	10	10	No limit
Sodium (g)	2.4	2.4	No limit

[1] Dietary recommendations for post-menopausal women.

[2] Dietary recommendations for women of reproductive age

[3] Based on the mean of recommended 10-20 E% of energy intake, values above 20 E% were capped.

Abbreviations: NRF11.3, Nutrient rich foods index 11.3

3.5 DIFFERING METHODS IN THE ADIRA- AND PIRA-TRIALS

Most procedures were similar between the ADIRA- and the PIRA-trial during screening, but the recruitment differed in some key aspects. Most notable, in the PIRA-trial only females were recruited. Further, in PIRA acceptable age span was from 20 to 70 years, where as in ADIRA it was set from 18 to 75 years. There was also cut-off for BMI when recruiting patients to the PIRA-trial, so that no obese patients were included in the trial. No such BMI related criterion was present in the ADIRA-trial.

Furthermore, presumptive participants prescribed some specific drugs (such as IL-6 inhibitors) were excluded in the PIRA-trial. Some more subtle differences include the time span of food records; in PIRA 4-day food records were used, and in ADIRA 3-day food records were used. In ADIRA, body composition was measured by BIS-measurement by multiple operators in the fasted state, whereas in PIRA, DXA and BIS measurements were combined and measured by a single dedicated operator, in standardized clothing on a void bladder in the non-fasted state. In the ADIRA-trial, all patients with RA who resided in areas where home-delivery by the company mat.se was possible, were invited. In the PIRA-study, all patients with a diagnosis of RA, who received medical care at the Sahlgrenska University Hospital, were invited to participate.

3.6 STATISTICAL ANALYSES

When analyzing the outcomes in the ADIRA-trial, the main analysis was a linear ANCOVA mixed model regression analysis (**Table 9**). Each outcome was adjusted for the run-in value prior to each dietary period, other fixed variables were period (first or second) and treatment .(intervention or control). Each participant was included as random intercept. For each outcome residuals were inspected, and when needed, the outcome was transformed to comply with model assumptions.

Table 9. *Statistical methods used in the included papers*

Main outcomes				
	Paper I	**Paper II**	**Paper III**	**Paper IV**
linear ANCOVA mixed model	X	X	X	X
Confounder analysis	X	X	X	X
Interaction analysis	X	X	X	X
Linear regression analysis				X
Baseline comparisons				
Fisher’s exact test		X	X	X
Kruskal Wallis test				X
Mann Whitney test		X	X	

3.6.1 CARRY-OVER EFFECTS

Washout was deemed sufficient not to influence outcome in paper I, carry-over effects were however explored in paper II and III.

In paper II, carry-over effects were evaluated by testing the interaction between diet period (first or second) and treatment (intervention or control) in the ANCOVA linear mixed model for CRP and ESR, with a preset cutoff for P-values at < 0.20. This analysis did not indicate any carry-over effect.

In paper III, carry-over effect was tested by using students' t-test on delta fat free mass (FFM), comparing developments between first and second period , within control and within intervention diet periods, with a cut-off for P-values at < 0.05. This analysis did not indicate any significant carry-over effect.

3.6.2 CONFOUNDER ANALYSES

Confounder analysis in the ANCOVA mixed model was done in papers I, II and III. Any covariate exerting a change in effect estimate (Beta) >10% was included as a fixed effect in the ANCOVA linear regression mixed model. In paper I, II and III, the following variables were tested for confounding:

- Age
- Sex
- Body mass index (BMI)
- Nicotine use (yes/no)
- Dietary quality (index between 0 to 12)
- Educational level (index between 1 to 5).

Similarly, in paper IV, any of the following variables that altered the effect estimate (Beta) >10% in a crude model were included as covariate in the final cross sectional linear regression:

- Study site (ADIRA / PIRA)
- Age (in years)
- Biological sex (male / female)
- Physical activity (a simplified index ranging 1-3)

3.6.3 INTERACTION ANALYSES

Interaction analyses were done on dichotomized data (above or below median) in papers I and III, and in paper II it was tested for carry-over effects (**Table 10**). In paper IV, habitual nutritional quality index was tested modelled as a continuous variable against developments in health outcomes (Table 10).

Table 10. *Interaction analyses performed in the papers in this thesis*

	Paper I	**Paper II**	**Paper III**	**Paper IV**
Outcome variables	• Total cholesterol • LDL-C • HDL-C • TG	• CRP • ESR	• FFM • FM • Fat mass %	• APO-B100/APO-A1 quote • CRP • DAS28 • ESR • Fat mass % • Non-HDL-C
Variables tested for effect modification	1. BMI (normal weight vs above normal weight) 2. Coronary risk evaluation (SCORE 2015)[1] 3. Dietary quality index[3] 4. Dietary fiber intake[3] 5. Dietary fat intake[3]	1. Diet period (1 or 2)	1. Habitual dietary quality 2. Physical activity (questionnaire of intentional exercise and daily activity) 3. High FMI[2] 4. Low FFMI[2] 5. Low FFMI & high FMI[2] 6. HAQ 7. Age 8. Educational level (below or above 2-years senior high school) 9. Albumin concentration 10. Employment status (employed / not employed)	1. Habitual nutritional quality (NRF11.3 score at screening)

[1]Swedish version of the systematic coronary risk score 2015, which indicates the risk of death of CVD within the coming 10 years (3).
[2] Below 25th percentile for FFMI or above 75th percentile for FMI, compared to reference values from the general population (4).
[3] Based on food frequency questionnaires at screening, ranked according to an index proposed by Swedish Food Agency to measure dietary quality (8)

Abbreviations:
LDL-C, Low density lipoprotein-bound cholesterol; HDL-C, High density lipoprotein-bound cholesterol; TG, Triglycerides; CRP, C-reactive protein; ESR, Erythrocyte sedimentation rate; BMI, Body mass index; CRP, C-reactive protein; ESR, Erythrocyte sedimentation rate; FFM, Fat free mass; FM, Fat mass; FMI, Fat mass index; FFMI, Fat free mass index; HAQ, Health assessment questionnaire disability index; APO-B100, Apoliprotein-B100; APO-A1, Apolipoprotein-A1; CRP, C-reactive protein; DAS28, 28-joints disease activity score erythrocyte sedimentation rate; ESR, Erythrocyte sedimentation rate; Non-HDL-C, Non high density lipoprotein-bound cholesterol; NRF, Nutrient rich foods index.

3.6.4 PER PROTOCOL AND INTENTION TO TREAT ANALYSIS

A per protocol analysis is based on the participants who complete a given intervention with acceptable compliance. In an intention to treat analysis, all available data included in the analysis, typically this should include all patients randomized to an intervention. There is however some uncertainty on how to properly handle missing data in an intention to treat analysis. In this thesis, per protocol analysis has been used and results are presented both on 1) all participants where data were available, and 2) participants who completed the intervention with high compliance and without major changes in medication.

4 RESULTS

Overall, the results of this thesis indicated that blood lipid profile, apolipoprotein concentrations and inflammation were affected by dietary intevention in patients with RA. Body composition improved over time regardless of diet allocation. Habitual nutritional quality was not tied to any of the assessed outcomes a cross sectional analysis.

The main outcome of the ADIRA-trial, the effects on DAS28, has been published outside of this thesis (89). When using the same intention to treat and per protocol analyses as in the papers included in this thesis, the results on DAS28 are as follows. In the whole group (n = 47), there was no effect between the intervention and the control diet period on DAS28 (mean: –0.289; 95% CI: –0.652, 0.075; $P = 0.116$). When excluding participants with new or discontinued DMARD of glucocorticoid treatment (previously unpublished data), DAS28 was lower after intervention compared to after the control diet period (mean: -0.487; 95% CI: -0.841, -0.133; $P = 0.009$) (n=38). Similarly, when analyzing only those without these major changes in medication and with high compliance to both diet periods (n = 29) (previously unpublished data), DAS28 was lower after intervention compared to after the control diet period (mean: -0.505; 95% CI: -0.854, -0.156; $P = 0.006$).

4.1 STUDY POPULATION

In the ADIRA-trial, out of the 50 patients included, 47 completed one diet period and 44 completed both diet periods. Out of those completing at least one diet period, 77% were female, median age was 63 years. About three quarters of participants used a csDMARD, a third bDMARD and around one in eight no DMARD at all. A tenth of participants changed their DMARD treatment, and another tenth changed their glucocorticoid treatment during the trial. Most had a moderate (57%) or low (28%) disease activity, only a minority were in remission (6%) or had a high disease activity (9%).

In the PIRA-trial, 30 patients supplied sufficient information (food records and body composition measurements) without acute illness, and were thus included in the cross sectional analysis. Participants in PIRA were all females with a median age of 60 years. A large part of these participants was either in remission (43%) or had low disease activity (30%), fewer had a moderate (20%) or high (7%) disease activity.

4.2 PAPER I

In paper I, examining blood lipid profile as a proxy for cardiovascular risk in the ADIRA-trial, the intervention diet increased HDL-cholesterol and reduced non-HDL-cholesterol as well as TG concentration compared to control diet. The increase in HDL-bound cholesterol occurred within the lower density HDL-particles (density range of 1.063 - 1.112 kg/L), and changes in TG primarily occurred in VLDL- and IDL-particles. Notably, Apolipoprotein B100 decreased, and the APO-B100/APO-A1 ratio improved (**Figure 13**). Lastly, the fatty acid composition in plasma was altered between the intervention and control period; saturated fatty acids were comparatively lower, whereas mono- and polyunsaturated fatty acids increased.

There were also characteristics that modified effects between intervention compared to control; HDL-C increased only in those with low risk of cardiovascular death (according to SCORE 2015). TG were reduced only in those either overweight, with a low habitual fiber intake, or a low habitual diet quality at study start. Similarly, LDL-C was comparatively lower in those with a high habitual fiber intake.

4.3 PAPER II

In paper II, the effects on markers of inflammation in the ADIRA-trial was evaluated in participants without changed medication. There was no effect on CRP or ESR in the group as a whole. When removing those with low compliance and who did not complete both diet periods, ESR was lower after intervention compared to after control diet period (Figure 13) (mean –5.490 mm/1h, 95% CI –10.310, –0.669 $P = 0.027$) (n = 29).

In a multiplex assay on a subset of the participants (n = 26), several chemokines (C-X-C motif chemokine ligands (CXCL)-1, -5, and -6) and the protein TNFS14 was lower after intervention compared to after control diet period. When removing those with low compliance, only the chemokines CXCL-1 and -6 and the protein GDNF were lower after intervention compared to after control.

***re 13.** Changes in blood lipids, apoliproteins and markers of inflammation in the ADIRA-trial between intervention and control diet periods*

es displayed are changes between after intervention diet period compared to after control diet period. Analyzed by a linear mixed ANCOVA
l, with the same model and confounders as in each respective paper.

ntion to treat; all available data from participants completing 1 diet period.

protocol; completed ≥1 diet period without major changes in medication during the trial.

protocol; completed both diet periods without major changes in medication and with high compliance.

4.4 PAPER III

In paper III, evaluating changes in body composition in the ADIRA-trial, there was no difference between intervention and control in any measurement of body composition (**Figure 14**), but an improvement in both groups over time regardless of treatment was detected.

An interaction analysis revealed that employment status modified the response; those not working had significantly lower fat mass after the intervention diet period compared to after the control diet period. Those not working were different to the others in the trial in several ways at study start; they had a higher fat mass % and FMI at study start, a higher age, they were all female, they reported a higher nutritional quality and fewer were medicated with csDMARDs.

4.5 PAPER IV

Paper IV revealed that the nutritional quality differed significantly between the intervention diet and the control diet periods within participants in the ADIRA-trial. Most participants improved in nutritional quality on the intervention diet, whereas the response to the control diet was more mixed (**Figure 15**).

The pooled cross-sectional analyses, from data collected at screening in the ADIRA- and the PIRA-trial, indicated no relation between nutritional quality index and any of the health outcomes examined.

Figure 14. *Changes in measures of body composition in the ADIRA-trial between intervention and control diet periods*

Values displayed are changes between after intervention diet period compared to after control diet period. Analyzed by a linear mixed ANCOVA model, with the same model and confounders as in each respective paper.

Intention to treat; all available data from participants completing 1 diet period.

Per protocol; completed ≥ 1 diet period without major changes in medication during the trial.

Per protocol; completed both diet periods without major changes in medication and with high compliance.

Figure 15. *Developments in nutritional quality during the control and the intervention diet periods*

5 DISCUSSION

The effect on DAS28 is published outside of this thesis, but will be clarified briefly. In the published paper on DAS28 (89), there was a significant effect in a Wilcoxon signed rank analysis comparing DAS28 after intervention to after control (n = 44). However, there was no effect in an ANCOVA linear mixed model on the group as a whole (n = 47), nor was there any effect when including only data from participants with unchanged DMARD and glucocorticoid treatment (n = 25). In comparison, in the papers that this thesis is based upon, participants were excluded from analysis only if completely discontinuing or starting on new DMARD or glucocorticoid treatment, ignoring changes in dosage or timing. Consequently, this renders a larger sample size. When using the same per protocol analyses as in the papers included in this thesis, DAS28 was lower after intervention compared to after the control diet period, highlighting both the potent effects of anti-rheumatic medications, and the impact of participant selection criteria.

5.1 CARDIOVASCULAR RISK, PAPER I

In the ADIRA-trial, we found that TG- and non-HDL-C concentrations decreased, HDL-C increased and the Apo-B100/A1 quote improved after intervention compared to after control diet (90). Our data do not allow us to draw any conclusion on functional properties of lipoprotein particles, but we can interpret the changes in lipid levels and apolipoproteins.

These changes in blood lipids seen in the ADIRA-trial could be compared to those in the largest RCT to date (n=7447) aimed at examining the effects of a Mediterranean diet supplemented with either extra virgin olive oil or nuts, the *Prevención con Dieta Mediterránea* (PREDIMED) trial (91). This study has been criticized on the basis that it could be seen as the control participants also consuming a type of Mediterranean diet (92). Still, in an intention to treat analysis in the Mediterranean diet group supplemented with nuts (the intervention most similar to ADIRA), a reduced hazard ratio of cardiovascular events (myocardial infarction, stroke or CVD death) of 0.72 (95% CI; 0.54 – 0.95) was found (91). An interim report about 12 weeks into PREDIMED revealed increased HDL-C (mean: 0.04 mmol/L, 95% CI: 0.01, 0.07) (93), lower than the effect estimate seen in the ADIRA-trial of (Mean: 0.074 mmol/L, 95% CI: 0.000, 0.148) (93). However, this report also saw decreased glucose and insulin concentrations as well as a reduction in blood pressure. Thus, the effects in the PREDIMED-trial are not entirely comparable to the results of the ADIRA-trial, but lends the possibility to hypothesize that a

prolonged intervention similar to the ADIRA-trial could reduce cardiovascular events.

A recent large cohort study investigated the importance of LDL-C, non-HDL-C, TG concentrations in relation to APO-B on risk of myocardial infarction (53). In this study, neither non-HDL-C nor TG concentrations were associated with cardiovascular risk when adjusted for Apo-B concentrations. This was true both for primary prevention (n=389529) and for secondary prevention in patients with established atherosclerosis (n=40430).

A previous systematic review found a decreased risk ratio of 7% (RR: 0.93, 95% CI: 0.94, 0.99) for cardiovascular death by each decrease in 10 mg/dL of APO-B (94), although the effect was limited to interventions that increased expression of the LDL-receptor. The between-diet period effect seen in the ADIRA-trial on APO-B100 was about 4 mg/dL (mean: -4.455, 95% CI: -3.505, 6.846) (90), suggesting that the gap between the two diets conferred a modest change in cardiovascular risk.

The mechanism behind the effect on blood lipid levels seen in the ADIRA-trial is not obvious, and the data do not allow for mechanistic conclusions. The intervention diet was relatively higher than the control diet in omega-3 fatty acids, which have previously (especially docosahexaenoic acid) been linked to decreased lipogenesis and increase beta-oxidation of fatty acids (75). Furthermore, the control diet was, relative to the intervention diet, high in saturated fatty acids, which can downregulate LDL-receptor expression and activity (95). The intervention diet was also high in fiber; a current theory is that higher fiber intake decreases blood cholesterol by binding to bile acids in the intestine as well as increases formation of short chain fatty acids in the colon, which might reduce circulatory cholesterol by lowered hepatic synthesis (96). The probiotic supplement used during the intervention diet might also have reduced the reuptake of cholesterol (97).

While significant effects were seen on markers indicating changes in blood lipid transportation, the behavior of HDL- and LDL-particles stretch beyond transporting lipids. As recently reviewed by Feingold et al. (98), smaller LDL-particles have a lower affinity to the LDL-receptor, thereby allowing for longer time in circulation. Additionally, the smaller LDL-particles more easily infiltrate the arterial walls and are more susceptible to oxidation. The HDL particle also takes part in a range of mechanisms in addition to mere lipid transport, such as antioxidant, inflammatory and antithrombotic processes (99).

As an example, previous large scale RCT studies on cholesterol ester transfer protein (CETP) inhibitors, which raise HDL-C by blocking transportation of cholesterol to Apo-B containing lipoproteins (essentially inhibiting some of the HDL particles' functions) have generated either unfavorable (100-103), or ambiguous results (104) on the risk of developing CVD. Assessing lipoprotein particle functionality or size was not available when handling data from the ADIRA-trial, thus, it might be worthwhile to bear in mind that we might not have captured the complete impact on CVD risk.

Another major factor for CVD is heightened blood pressure; no effect on blood pressure was found in the ADIRA-trial. However, neither the intervention nor the control diet was designed to alter sodium intake, which is the main dietary factor related to blood pressure.

5.2 INFLAMMATION, PAPER II

When examining biomarkers of inflammation, we saw a significant reduction in ESR among those who reported to have completed both diet periods with high compliance, but only a nonsignificant trend was seen in the whole group, and no effect on CRP-concentration (105).

Most previous dietary intervention studies in patients with RA that indicate beneficial effects on ESR or CRP have done so during concurrent weight loss (10, 12, 15, 16), or have not reported data on weight change (6, 9). To the best of our knowledge, only one previous trial, investigating the response to a hypoallergenic diet, found lowered inflammation when controlling for weight changes (13). An important strength of the ADIRA-trial is thus that no significant weight change occurred during or between the dietary intervention periods.

The available data do not allow for a mechanistic explanation of these results, nor does it allow us to pinpoint effects to specific foods since the intervention was given as a portfolio diet. However, several key differences in design between the intervention diet and control diet could have influenced the results such as dietary fatty acid, fiber-, phytochemical- and wholegrain intake.

The intervention diet was specifically higher in omega-3 fatty acid from marine foods compared to the control diet, which could have led to the formation of less proinflammatory eicosanoids. Chronic inflammation is also linked with oxidative stress, and prolonged oxidative stress can lead to lipid oxidation, lipoprotein dysfunction and cell damage. Higher levels or reactive oxygen species, deoxyribonucleic acid (DNA) damage and lipid peroxidation have

been found in patients with RA compared to healthy controls (106). The intervention diet in ADIRA was markedly higher in phytochemicals. Although we lack data on oxidative status of participants, it is possible that the difference in phytochemicals between the two diets conferred a benefit during the intervention diet through a reduction in oxidative stress.

The difference in fiber content might also have affected inflammation. This has been indicated in an uncontrolled trial on patients with RA that saw increased short-chain fatty acids, a product from the intestinal microflora, in serum, coupled with decreased proinflammatory cytokines, during concurrent fiber-bar supplementation (107).

Results from the Olink multiplex assay indicated that effects were found in CXCL-1, and -6, and depending on participant selection CXCL-5, TNFSF14 or GDNF, without correction for multiple hypothesis testing. The number of hypothesis tests in the multiplex assay makes it highly unlikely that not at least some of the results are due to type-1 errors. However, CXCL-1, -5 and -6 are proteins that are related in structure and function. It could be reasonable to postulate that the effects seen in these might reflect physiological changes. CXCL-1, -5 and -6 belong to the cytokine class called chemokines, that direct movement of leukocytes to a site of infection or tissue damage. Previous data from diet interventions on these chemokines are rare. Animal studies have demonstrated increased concentrations of CXCL-1 and -5 in serum of overweight and in hyperglycemic conditions (108), indicating that these proteins are involved in both inflammation and metabolic regulation.

5.3 BODY COMPOSITION, PAPER III

When examining changes in body composition, we demonstrated that body composition among patients with RA can indeed be affected by participating in a dietary intervention study, without affecting body weight (105). The causality of improvements over time regardless of treatment is not clear, but in line with previous results from a study on dietary supplements in patients with RA (81). There is no possibility to separate dietary effects from potential changes in other lifestyle related behavior (e.g., physical activity or sleep), even when were not actively encouraged during the study.

We further identified an interaction in that we saw that a subgroup of not employed participants actually responded differently to the intervention and control diet periods, albeit the reasons for this remain elusive. At baseline, the non-employed group was significantly different in regard to body fatness, age, sex, medication and dietary intake. Likely, employment status could be a proxy

for several yet unknown factors. One could speculate that the higher body fatness in this subgroup made them more open to lose fat, and that the intervention diet, through less refined and fiber rich foods, could have affected satiety and nutrient metabolism, thereby reducing body fatness.

5.4 NUTRITIONAL QUALITY INDEX, PAPER IV

When examining health outcomes in a cross sectional analysis on pooled data from the ADIRA- and PIRA-trials, no relation to the nutritional quality index was revealed in any outcome. It could be that this patient group were already sufficiently nourished, or it could be that the index was not capable of capturing the dietary intake well enough. One needs to keep in mind that the sample size was rather small, and that self-reported 3-4 days food records might not accurately enough capture habitual dietary intake. The accuracy of FFM assessed by BIS is also not very high (109), and it is plausible to assume that not all variation in FFM is captured by this method in a cross-sectional setting.

When assessing the developments within the ADIRA-trial, it was clear that nutritional quality of the intervention diet differed to the control diet, indicating that at least in terms of self-reported intake, the dietary intervention and control diet were successfully implemented. Some previous studies, such as the dietary intervention by Skoldstam et al. (16), instead of using an active comparator, recruited selectively based on habitual dietary intake in order to achieve a control group with a habitual diet resembling a typical western diet. A drawback with a passive control design, however, is the risk of introducing variations between intervention and control beyond the designed altered dietary intake. In this aspect, the ADIRA-trial likely more accurately compared two different dietary regimens.

As further outlined in paper IV, there were several interactions between habitual nutritional quality and health outcomes in the ADIRA-trial. In a dietary intervention trial, it would seem plausible to expect a varying response dependent on the baseline dietary intake. However, none of the subgroup analyses, based on habitual nutritional quality, indicated significant results between the intervention and the control diet. Thus, it remains hard to draw any conclusions.

While most individuals improved in nutritional quality during the intervention diet period, the response to the control diet was more diverse (Figure 15). Further, there was at least one outlier who strongly decreased in nutritional quality during the intervention diet periods. In this particular case, the calculated energy intake averaged at 600 kcal/day after the intervention diet

period, a decrease of about 1000 kcal/day compared to pre intervention, rendering the daily intake of virtually all nutrients below recommended levels. Measuring dietary intake in free living individuals is rarely an exact science, as another example, there was no correlation between energy intake and weight change during the diet periods (data not shown). Under-reporting coupled with variations in daily intake are likely important factors behind this phenomenon.

An important limitation that applies specifically to paper IV is the handling of the NRF11.3 index. This index was originally developed based on nutrient density per standardized serving size or per 100 kcal (86). The validation and modification of this index in a large Swedish cohort was based on Nordic recommendations on daily intake of nutrients, compared to energy-normalized daily nutrient intake as estimated in food frequency questionnaires (87). In paper IV, the analysis of developments during the ADIRA-trial is based on the same index of nutrients identified as important in relation to longevity by Strid et al. (87), but instead of energy density, absolute intake was assessed. Arguably, this approach answers a slightly different question than an energy normalized calculation would do. However, in the cross sectional analyses, the nutritional quality index was not related to any health outcomes, regardless if calculated as nutrient density or as nutrient adequacy adjusted for energy intake.

5.5 THE ADIRA STUDY DESIGN

5.5.1 DIETARY INTERVENTION FOODS

One key difference between the ADIRA-trial and most other Mediterranean-like diet interventions, is that no extra virgin olive oil was used. Extra virgin olive oil is suggested to induce a range of anti-inflammatory effects due to its high content of polyphenols (110), and the European Food Safety Authority has approved a health claim that extra virgin olive oil protects LDL-particles from oxidation (111). In contrast, canola oil, produced locally in Sweden, was used in the ADIRA-trial. Even though data on immunomodulatory potential of canola oil are rather scarce, it does not appear to be comparable to refined olive oil (112), or extra virgin olive oil (113) in other patient populations. The fatty acid composition, including omega-3 fatty acids, is however excellent and there are documented positive effects on the blood lipid profile from intake of canola oil (114). This component of the intervention could thus in part confer the beneficial effect on blood lipids observed in the ADIRA-trial.

The probiotic strain used in the ADIRA-trial, *Lactobacillus plantarum 299v/ DSM 9843*, has not previously been tried on patients with RA. However, in an uncontrolled trial on men with coronary artery disease, a decreased level of proinflammatory cytokines (115) and gene expressions in PBMCs (116) was noted. Naturally, the lack of a proper control group and differing patient characteristics call for a cautious interpretation. Previous interventions with probiotic strains indicating beneficial effects on patients with RA include *Bifidobacterium bifidum, Lactobacillus acidophilus and Lactobacillus casei* combined (117, 118), as well as *Lactobacillus casei 01 (119)* who all reduced CRP. In the ADIRA-trial, we lack data to specifically confer effects to the probiotic supplementation.

5.5.2 PARTICIPANTS

The participants in the ADIRA-trial were mostly older females, while incidence of RA is increased in this group, the results might not be representable to different patient groups. Another example is socioeconomic factors; educational level appears to relate to health outcome and life expectancy among patients with RA (120). In the ADIRA-trial, educational level was generally high; 49% of those who completed at least one diet period had a university level education. This is similar to the general population average of 44% (121), but perhaps slightly higher than the average among females in a similar age range where 42% has a university level education (122). These factors could limit the external validity.

5.5.3 RECRUITMENT

Another parameter in the ADIRA-trial potentially affecting external validity is the recruitment process; 15% of those invited by letter agreed to participate, and a selection bias is likely. One could expect that participants that reply to letters of invitation have an above-average interest in diet and health-encouraging lifestyle approaches. It could be postulated that this selection also predispose to a higher compliance than that of the average patient with RA. This situation warrants the use of an active comparator design in order to compare the two different dietary approaches. This does however also hinder us from speculating on the effect of a dietary intervention compared to the habitual diet of patients with RA in a clinical setting.

The mean DAS28 value of the 50 participants entering the ADIRA-trial was at 3.8. There are no systematic national statistics on the median value of DAS28 in patients with RA in Sweden (personal communication, SRQ), but in general, a value above 3.2 instigates pharmacological intervention with the aim of reaching remission (123). It could thus seem questionable to include patients that needed medical intervention. However, a rheumatologist examined each patient at inclusion and only those assessed as adequately medicated were included in the study.

In general, as described in a wide range of datasets, for extreme measurements within a variable that can vary, values tend to normalize over time, a statistical concept referred to as regression to the mean (124). The ADIRA-trial recruited patients that were estimated to have a DAS28 score of at least 2.6, which indeed is a variable that can vary over time. The rationale was to recruit patients with ongoing inflammation, so as to be able to produce a decrease. This approach also has a downside; while the differences between intervention and control diet periods in the group as a whole were not significant (89), there was indeed an improvement in DAS28 between pre and post values regardless of treatment during the first diet period (period 1 mean difference; -0.42, $P < 0.001$, n= 47), but no such improvement was seen in the second diet period (period 2 mean difference -0.01, $P = 0.947$, n = 44), analyzed by a paired t-test. This pattern is compatible with the concept of a regression to the mean effect and could affect all outcomes analyzed. However, the randomized study design and the statistical analysis where only the differences between dietary treatments are interpreted, adjusted for diet period, should counteract this phenomenon in the published papers. Further, while ESR concentration is a significant factor in DAS28, the improvements during this first period was mainly related to improvements in tender joints (data not shown). This is a

subjective factor that perhaps could be affected by psychological well being, which could have improved by being part of a clinical trial.

5.5.4 STATISTICAL CONSIDERATIONS

All the articles in this thesis have been produced without correction for multiple hypothesis tests, and as such are mainly to be viewed as indicative and exploratory rather than conclusive; more studies are needed to replicate and verify our findings.

In paper II, there was an exploratory analysis on a wide range of biomarkers related to inflammation. There was a hypothesis that IL-6 or TNF could be affected by the intervention, but there was no hypothesis that chemokines would be affected. While the results seem plausible, the multi-testing approach needs to be considered. The chance of type-1 error can be computed by $(1 - (1-\alpha)^n$, where n is the number of hypothesis tests performed. In the case of the multiplex assay, 72 tests were done in two group selections, totaling in 144 tests, each with an α-value of 0.05. This translates to about 99.9% probability of finding at least one marker that is significantly different by pure chance (**Figure 16**).

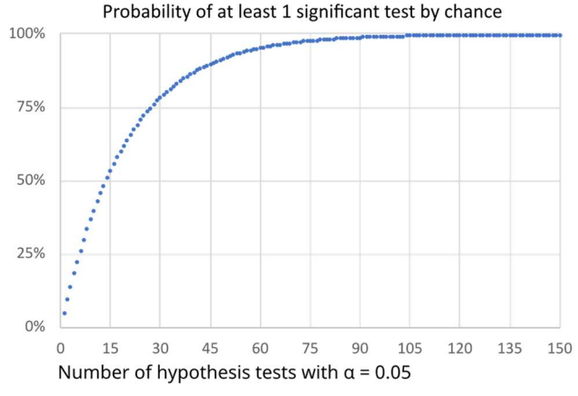

***Figure 16**. The risk of type-1 error by increasing number of hypothesis tests*

There are several methods to counteract multiple hypothesis tests, and the most popular is probably the Bonferroni correction. This method suggests dividing α by the number of hypothesis tests. The most appropriate dataset in this thesis to apply correction for multiple hypothesis tests is arguable the multiplex assay performed in paper II. By using a Bonferroni correction, the significance level would be set to $\alpha < 0.000347$, and no results from the multiplex assay would be deemed significant. However, it has also been argued that the Bonferroni

correction answers a largely irrelevant question, since focus shifts from each individual parameter to an assessment of whether two groups are identical in all possible aspects (125). In the papers included in this thesis, it was decided to not correct for multiple hypothesis test in order not to be overly conservative and introduce type-2 errors, and hence results need to be interpreted accordingly.

Another common line of thought seems to be that it is harder to find significant findings in smaller datasets, thereby concluding that significant results from smaller datasets are robust. However, while it might be harder to find true differences in small datasets, the risk of chance findings from clustering by chance also increases with a limited number of participants. It would be reasonable to replicate our results with appropriate statistical power for each outcome of interest. In the ADIRA-trial, the power calculation was performed with the aim of investigating DAS28, an outcome variable not included in the papers this thesis is based upon; all outcomes included are secondary outcomes. To verify our results, a larger study sample would likely be needed.

Still, there is a scarcity of dietary intervention studies in patients with RA. The robust study design of the ADIRA-trial, in an area where not many clinical trials have been performed, motivates for exploration of secondary outcomes despite the risk of introducing type-1 errors.

5.5.5 MEASUREMENTS OF OUTCOMES

In the ADIRA-trial, measurements were taken before and after each dietary period, which allowed for an adequate evaluation of effects. It would also have been interesting to take multiple measurements over time, analyzing the trend within each diet period as repeated correlated measures or perhaps as area under the curve. With this approach, the statistical analysis could have gained in strength, and it would have been possible to perform mediator analyses.

There was no objective measure of individual compliance in the ADIRA-trial; interviews with participants were performed mid-period and food records were assessed before and after each diet period. Interview with study staff about one´s compliance to the study protocol could lead to overly positive results. Food records are subject to two phases of bias; first by the participant to accurately report intake and to maintain a habitual diet while recording everything eaten, secondly by the dietitian to interpret types and amounts of foods when not precisely detailed. It would thus be interesting to analyze developments in objective biomarkers of compliance and response, such as for example alkylresorcinols for whole grain intake (126), or fatty acid

composition of red blood cells, or carotenoids for vegetable intake. Surely, some of these markers will be revealed in forthcoming articles based on the ADIRA-trial. However, we did already assess changes in plasma fatty acid composition, which indicated that, on a group level, the dietary regimens significantly affected the composition of fatty acids in the circulation.

Even though we believe that the washout period was sufficient in length to facilitate a normalization of habitual intake, metabolic traces of a nutritional intervention could well span for a prolonged time period. As an example, TG age in human adipocytes has a turnover rate of approximately 1.6 years in homeostatic conditions (127). It is thus possible that stronger effects could have been presented if ADIRA had a parallel study design, eliminating any potential for carry-over effects. However, this would require a larger sample size. In the ADIRA-trial, all eligible participants in the region were invited, as such it would not have been plausible to employ a parallel design without incorporating several study sites or simplifying the study protocol. In order to reduce interindividual variation and thereby maximize statistical power in the ADIRA-trial, without employing a multi-center design, a cross-over design was chosen.

5.6 CONCLUSION & FUTURE PERSPECTIVES

Diet matters. Dietary intake can modify CVD risk profile, biomarkers of inflammation, and body composition in patients with RA. Even though the data presented in this thesis suffer from multiple hypothesis tests, it reveals new novel findings, and indications of efficacy that merits further replication and longer term evaluation.

In terms of determining the long term benefit of dietary optimization for patients with RA, an intervention in the clinical setting could be done. For example, newly diagnosed patients could be invited and randomized to dietary counselling or control in a parallel design. This would allow for a proper evaluation of the feasibility of upholding compliance over time, and long term outcomes.

In addition to evaluating the long term consequences of dietary modification in patients with RA, it would also be interesting to shed light on the acute effects of dietary intake in the postprandial phase. During screening, several patients in the ADIRA- and PIRA-trials reported feeling worse after intake of certain foods (data not published), which they habitually avoided. It would thus be interesting to evaluate the postprandial state after intake of these types of

foods. Arguable, the postprandial state might be more representable to the habitual state of humans.

Furthermore, in addition to examining dietary effects during weight stability, it would also be interesting to perform a study aimed at optimizing body composition in obese patients with RA. Ideally by a combined diet and exercise regimen. This would allow for determining the clinical relevance of metabolic normalization, perhaps in comparison to the effect of pharmacological interventions.

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