Running title

Diet and inflammation in rheumatoid arthritis

Title

A proposed anti-inflammatory diet reduces inflammation in compliant weight stable patients with rheumatoid arthritis in a randomized controlled crossover trial

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Sources of support: Granted by funds from the Swedish government under the ALF agreement (Award number ALFGBG-716341), Swedish Research Council for Health, Working Life and Welfare (FORTE) (Award number 2017-00318), the Inger Bendix Foundation, the Lennander Foundation, Sahlgrenska University Hospital Foundations and the Gothenburg Region Foundation for Rheumatology Research (GSFR).

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Conflict of interest: Erik Hulander, Linnea Bärebring, Anna Turesson Wadell, Inger Gjertsson, Philip C Calder, Anna Winkvist and Helen M Lindqvist all declare no conflicts of interest.

Philip C Calder is an Associate Editor on The Journal of Nutrition and played no role in the Journal's evaluation of the manuscript

Word count: 3659.

Number of figures to print: 2

Number of tables to print: 2

Supplementary data submitted: Supplemental Figure 1, Supplemental Table 1-3.

Abbreviations

Anti-inflammatory Diet In Rheumatoid Arthritis	ADIRA
Biological disease modifying anti-rheumatic drug	bDMARD
Conventional synthetic disease modifying anti-rheumatic drug	csDMARD
C-C motif chemokine 28	CCL28
C-C motif chemokine 23	CCL23
C-reactive protein	CRP
C-X-C motif chemokine 1	CXCL1
C-X-C motif chemokine 5	CXCL5
C-X-C motif chemokine 6	CXCL6
Disease modifying anti-rheumatic drug	DMARD
Disease activity score 28 joints erythrocyte sedimentation rate	DAS28-ESR
Erythrocyte sedimentation rate	ESR
Glial cell line-derived neurotrophic factor	GDNF
Health assessment questionnaire	HAQ
Rheumatoid arthritis	RA
Tumor necrosis factor ligand superfamily member 14	TNFSF14

Abstract

Background: It is unclear to what extent adjuvant dietary intervention can influence inflammation in Rheumatoid arthritis (RA).

Objectives: The objective was to assess the effects of dietary manipulation on inflammation in patients with RA.

Methods: In a crossover design, participants (n=50, 78% females, median body mass index 27 kg/m^2, median age 63 years) were randomized to begin with either a 10-week portfolio diet of proposed anti-inflammatory foods (i.e. a high intake of fatty fish, wholegrains, fruits, nuts and berries) or a control diet resembling a Western diet with a 4-months washout in between. This report evaluates the secondary outcome markers of inflammation among participants with stable medication. Analyses were performed using a linear mixed ANCOVA model.

Results: There were no significant effects on CRP or ESR in the group as a whole. In those with high compliance (n = 29), changes in ESR within the intervention diet period differed significantly compared to within the control diet period (mean: -5.490, 95% CI: -10.310, -0.669; $P = 0.027$). During the intervention diet period, there were lowered serum concentrations of C-X-C motif ligand (CXCL)1 (mean: -0.268, 95% CI: -0.452, -0.084, $P = 0.006$), CXCL5 (mean: -0.278, 95% CI: -0.530, -0.026 $P = 0.031$), CXCL6 (mean: -0.251, 95% CI: -0.433, -0.069, $P = 0.009$) and tumor necrosis factor ligand superfamily member 14 (TNFSF14) (mean: -0.139, 95% CI: -0.275, -0.002, $P = 0.047$) compared to changes within the control diet period.

Conclusion: A proposed anti-inflammatory diet likely reduced systemic inflammation, as indicated by a decreased ESR in those who completed the study with high compliance

(n=29). These findings warrant further studies to validate our results, and to evaluate the clinical relevance of changes in CXCL1, CXCL5, CXCL6 and TNFSF14 in patients with RA.

This trial was registered at clinicaltrials.gov as NCT02941055.

Keywords

Mesh term	**Mesh ID**
Inflammation	D007249
Diet, Mediterranean	D038441
Diet, Western	D066273
Cross-Over Studies	D018592
Arthritis, Rheumatoid	D001172

Introduction

Around 5% of the population suffers from an autoimmune disease (1). A common feature of autoimmune diseases is a life-long disabling effect on afflicted individuals, with an etiology that is largely unknown. Rheumatoid arthritis (RA), one of the most common autoimmune diseases, affects approximately 0.5 – 1% of the population in North America and Europe, though prevalence varies by geographical region (2). Symptoms of RA primarily include pain, swelling and reduced function in peripheral joints. The chronic activation of inflammatory pathways also leads to a state of elevated systemic inflammation, which can increase the risk of co-morbidities. Although pharmacological treatment of RA has improved substantially during the past decades, there is no cure and many patients still experience incomplete treatment response (3). A fear of side effects related to medical treatment and a belief that environmental factors modulate disease development and activity have been described for patients with RA (4), causing many patients to experiment with their lifestyle. In a Finnish survey, 50% of patients changed their diet after a RA diagnosis, many believing red meat and animal fats to be detrimental (5). There is growing interest to understand the role of diet as a modulator of inflammatory activity. Several attempts have been made to determine beneficial foods and dietary patterns, such as the dietary inflammatory index (6) and the Mediterranean diet score (7). There is also some evidence from clinical trials on patients with RA that fish oil supplementation, fasting, and a Mediterranean-like diet pattern could reduce measures of disease activity and inflammation (8).

The rationale and primary aim of this study was to investigate whether a portfolio diet (compared to a typical Western diet), combining potential anti-inflammatory foods, could beneficially alter biomarkers of inflammation in patients with RA. We have previously demonstrated the effect of this diet on Disease Activity Score 28 joints erythrocyte sedimentation rate (DAS28-ESR), a recognized clinically relevant composite index of

59 subjective and objective markers of disease activity (9). Here, we report the effects of the
60 portfolio diet on biological markers of inflammation as secondary outcomes.

Methods

This report is an analysis of secondary outcomes; the main outcome of the Anti-inflammatory diet in Rheumatoid Arthritis (ADIRA) trial was DAS28-ESR, for which results have been published (9).

Ethical statement

This study was approved by the regional ethical review board in Gothenburg (976-16 and T519-17) and registered on ClinicalTrials.gov (NCT02941055). Participants provided signed informed consent prior to enrollment and all procedures were performed according to the Helsinki Declaration.

Recruitment

Patients diagnosed with RA according to 1987 American College of Rheumatology and 2010 American College of Rheumatology / European League Against Rheumatism criteria (10) listed at Sahlgrenska University Hospital (Gothenburg, Sweden) were selected from the Swedish Rheumatology Quality Register (n = 1091). Those who resided in areas in the Gothenburg region where home delivery of food was possible (n = 774) were invited to participate. In total, 113 patients volunteered to take part in the study. After initial contact, 47 were deemed not to fulfill inclusion criteria, thus 66 volunteers were screened for inclusion and out of those, 50 were included in the study. Inclusion criteria were DAS28-ESR $\geq$ 2.6, unchanged disease modifying anti-rheumatic drug (DMARD) medication during the previous eight weeks, 18-75 years of age, and at least two years disease duration. Life threatening diseases, pregnancy or lactation, food allergies to components in the dietary intervention, inability to communicate verbally and inability to understand study instructions were exclusion criteria.

Study design

A crossover design was chosen to minimize inter-individual variation in a heterogeneous population of patients with RA. Study staff randomized the participants (allocation ratio 1:1) to begin with either intervention or control diet using a computer-generated list. The 10-week diet periods were separated by a 4-month washout period. The study ran in two batches, commencing in February 2017 and August 2017, respectively.

Dietary intervention

The dietary intervention has been described in detail elsewhere (9, 11, 12). In brief, the intervention diet had a nutritional profile similar to the Mediterranean diet, being rich in whole grains and fatty fish, enriched with probiotics and high in phytochemicals through legumes, nuts, fruits, berries and vegetables. However, instead of olive oil, canola oil was used. Advice was given to limit red meat to ≤ 3 times per week and keep fruit, berry and vegetable intake to ≥ 5 portions daily and to choose whole grain products. Low-fat dairy products and use of margarine and vegetable oils for cooking were encouraged.

The control diet resembled a Western diet, being high in refined grains, red meat and chicken, and low in fruit and vegetables. As snacks, protein bars and protein puddings as well as quark were included. Participants were advised to keep fish intake to ≤ 1 and red meat ≥ 5 times per week. Advice was also given to keep intake of fruits, berries and vegetables to ≤ 5 portions daily as well to avoid probiotic products. Whole-fat dairy products and use of butter for cooking were encouraged.

Participants received home-delivery of groceries with recipes and menus, which accounted for an intake of approximately 1100 kcal/day for 5 days per week, designed to cover approximately half of the daily energy intake. Study staff urged participants to keep weight-stable and provided foods were isocaloric between diets. In an attempt to blind

participants, study staff consistently referred to the intervention diet as “fiber diet”, and control diet as “protein diet” in all communications.

Data collection

At screening, participants filled out questionnaires on medication usage, age and demographic background as well as the RA-specific Health assessment questionnaire (HAQ) (13). Waist-to-hip ratio and height was measured to the closest 0.5 cm and non-fasting blood samples were collected by venipuncture for analysis of Erythrocyte sedimentation rate (ESR) and concentration of C-reactive protein (CRP). Measurements of CRP and ESR from screening were used as baseline values for the first diet period, but subsequent blood samples (and all serum samples) were collected in the fasted state. Before and after each diet period, blood samples were collected by venipuncture, dietary intake was recorded in 3-day food records, weight was measured to the nearest 0.1 kg and 1 kg was subtracted to account for clothing. To estimate disease activity by DAS28-ESR, joint examination was performed by nurses experienced in rheumatology.

During each dietary period, participants were urged to record any changes in medications. Furthermore, study staff interviewed participants by telephone asking if and to what extent each study meal had been consumed during the past week and calculated a score; in whole (2 points), in part (1 points) or not at all (0 points). This yielded a numerical score for each participant from 0-30. Participants reaching a score of over 24 points (> 80%) were considered compliant.

Laboratory analyses

Concentration of CRP and ESR were measured by routine analysis in fresh samples at Sahlgrenska University Hospital (Gothenburg, Sweden).

Serum was separated by leaving blood samples for 5 minutes in room temperature, 30 minutes in the refrigerator, and then centrifuging for 10 minutes at 2594 x *g*. Serum samples were stored at -80°C until analysis, but thawed once to prepare aliquots for external analyses. Because sampling procedure affects the analysis of inflammation-related proteins in serum, only blood samples that were handled according to our most strict protocol, and available from all study visits, were analyzed for inflammation-related proteins. This included samples from 32 subjects.

To quantify inflammation-related proteins, a multiplex assay measuring relative concentrations of 92 inflammation-related proteins was deployed and analyzed externally by Olink Proteomics AB, using the Olink® Target 96 Inflammation panel (Olink Proteomics AB, Uppsala, Sweden), as described elsewhere (14). In brief, pairs of oligonucleotide-labeled antibody probes bind to their targeted protein, and if the two probes are brought in close proximity the oligonucleotides will hybridize in a pair-wise manner. The addition of a DNA polymerase leads to a proximity-dependent DNA polymerization event, generating a unique PCR target sequence. The resulting DNA sequence is subsequently detected and quantified using a microfluidic real-time PCR instrument (Biomark HD, Fluidigm). Data are then quality controlled and normalized using an internal extension control and an inter-plate control, to adjust for intra- and inter-run variation. The final assay read-out is presented as a normalized protein expression value, which is an arbitrary unit on a log2-scale where a high value corresponds to a higher protein expression. If any of the internal controls deviates more than ± 0.3 from the plate median, the sample fails quality control. All assay validation data are available on the manufacturer's website (www.olink.com). Data from the Olink analysis were included only on proteins where at least 90% of the samples had results above the valid lower limit of detection and only on samples that passed quality control. This limited the quantification to 72 inflammation-related proteins (**Supplemental Table 1**).

Statistical analysis

Statistical analysis was performed by a linear mixed ANCOVA model using IBM SPSS version 25. Fixed variables were dietary treatment (intervention or control diet), period (first or second diet period), body mass index (BMI), and baseline value of each outcome variable. Individual participants were included as random effects. Residuals were inspected and variables with skewed distributions were transformed in order to comply with model assumptions. There was no correction for multiple hypothesis tests. The power analysis of the ADIRA trial was performed on the primary outcome DAS28-ESR. In order to detect a change of 0.6 units in DAS28-ESR with 90% power and $\alpha = 0.05$, a sample size of 38 patients was needed and to account for dropouts 50 patients were recruited.

In order to avoid distortion of results due to changes in anti-inflammatory medication, participants who completely stopped or started on a new DMARD or glucocorticoid treatment during the diet periods were excluded from analysis. In total, 38 participants completed at least one diet period (37 completed the intervention diet, 37 completed the control diet) without discontinued or new DMARD or glucocorticoid treatment (**Figure 1**). Quantification of inflammation-related proteins in the multiplex assay was performed on samples handled according to the strictest protocol; such samples were available from 26 participants who completed both diet periods.

Sensitivity analysis

In an attempt to further explore the results, a sensitivity analysis was performed. In addition to excluding those who stopped or started on a new DMARD or glucocorticoid treatment, only those who completed both diet periods and reported high compliance during both diet periods (> 80%) were included in this analysis. Excluding participants with low compliance and those who discontinued any of the diet periods, yielded 29 participants for

180 the analysis of ESR and CRP, and 20 participants for analysis of inflammation-related
181 proteins in the multiplex assay.

182 *Carry-over effect*

183 In order to examine carry-over effects, interaction between dietary treatment
184 (intervention or control) and diet period (1 or 2) for CRP and ESR were tested. There were no
185 significant interactions ($P > 0.20$) between diet period and treatment.

186 *Group selection bias*

187 In order to assess bias in group selections, baseline characteristics of participants were
188 compared between those included and not included in analyses. Those included in analysis
189 with the multiplex assay were compared to those not included, and participants who
190 completed both diet periods with high compliance without new or discontinued DMARD or
191 glucocorticoid treatment were compared to those who did not. Continuous variables were
192 compared using Mann-Whitney U-test while categorical variables were compared using
193 Fishers Exact test.

194

Results

Participants

Overall, three quarters of the participants were women and around half had a university-level education. The vast majority were non-smokers of European descent and over half were treated with a conventional synthetic disease modifying anti-rheumatic drug (csDMARD) and about a third with a biological disease modifying anti-rheumatic drug (bDMARD) (**Table 1**). A majority of participants were middle-aged or older, had a moderate disease activity (defined by DAS28-ESR between 3.2 and 5.1), and were either overweight or obese (Table 1).

Adverse effects

In the group as a whole (n = 38), there were 15 reports of gastrointestinal discomfort, with 11/15 during the intervention diet period. Among the patients where inflammation-related proteins were measured (n = 26), there were nine reports of gastrointestinal discomfort, of which 7/9 were during the intervention diet period.

Group selection bias

Participants without new or discontinued DMARD or glucocorticoid therapy who continued both diet periods with high compliance (n = 29), had lower waist-to-hip ratio (P = 0.006) and a higher educational level (P = 0.030), but did not otherwise differ from the rest of the participants (n = 18). Among those whose samples were selected for multiplex analysis (n = 32), leucocyte concentration was lower (P = 0.024) than the rest (n=15). Furthermore, percentage of energy intake from total and saturated fat was higher (P = 0.027 and P = 0.027 respectively) while percentage of energy intake from carbohydrates was lower (P = 0.040) in those included compared to those not included in the multiplex analysis.

218

219 **Effects of diet on clinically validated markers of inflammation**

220 There were no effects of diet on CRP ($P = 0.125$) or ESR ($P = 0.059$) in the main
221 analysis (**Table 2**). There was however a significant increase in ESR during the control diet
222 period. In the sensitivity analysis, ESR was lowered during the intervention diet period
223 compared to during the control diet period (mean between-periods difference: -5.490 mm/h,
224 95% CI -10.310, -0.669; $P = 0.027$).

225

226 **Exploratory analysis of biomarkers related to inflammation**

227 C-X-C motif chemokine ligand (CXCL)1, CXCL5, CXCL6 and tumor necrosis factor
228 ligand superfamily member 14 (TNFSF14) were significantly lowered during the intervention
229 diet period compared to during the control diet period in the main analysis (**Figure 2** and
230 **Supplemental Table 2**). No other significant between diet-period effects were identified
231 (Figure 2 and Supplemental Table 2).

232 In the sensitivity analyses, results for CXCL1 and CXCL6 remained significantly
233 lowered during the intervention diet period compared to during the control diet period
234 (**Supplemental Figure 1** and **Supplemental Table 3**). Additionally, glial cell line-derived
235 neurotrophic factor (GDNF) differed significantly between diet periods, with a lowered value
236 during the intervention diet period compared to during the control diet period (Supplemental
237 Figure 1 and Supplemental Table 3).

238

239

Discussion

This investigation examined the hypothesis that dietary manipulation, using a proposed anti-inflammatory portfolio diet, will further decrease inflammation in patients with RA during stable and adequate anti-rheumatic pharmacological treatment. To our knowledge, this is the most comprehensive analysis of effects on biomarkers of inflammation from dietary manipulation in patients with RA in a randomized controlled trial.

The clinically validated markers of inflammation (CRP and ESR) were unchanged by the diet in the main analysis, but among participants who reported high compliance and who completed both diet periods, a significant treatment effect on ESR was seen. This highlights controlling for compliance as a key priority in studies on effects of dietary intervention in humans.

ESR is a rather simple and readily available laboratory test that along with CRP is the recommended measure of acute phase reactants in clinical care of patients with RA (15). As recently reviewed, ESR is a non-specific marker of inflammation in general (16). The data in our trial do not permit us to draw any conclusions on the mechanism by which the treatment diet lowered ESR in this patient population. Several foods in the intervention diet might act in an anti-inflammatory manner. For example, omega-3 fatty acids from the fatty fish can act as a competitive substrate with arachidonic acid for the cyclooxygenase, lipoxygenase and cytochrome P450 enzymes yielding less inflammatory eicosanoids, and they may also act as substrates for synthesis of pro-resolving lipid mediators. In addition, a high intake of fruits, berries, vegetables, nuts and seeds containing phytochemicals may potentially dampen oxidative stress, which in turn could reduce general inflammatory activity. It is also possible that the higher fiber intake (through whole grains and less

263 processed foods) coupled with probiotics affected the microbiota and, increased the
264 production of short chain fatty acids, which could exert an anti-inflammatory effect (17).

265 Studies with similar dietary interventions in patients with RA are rare. McKellar *et*
266 *al*. used cooking classes as a method to reach a Mediterranean-like diet among participants in
267 socially deprived areas, but found no effects on inflammation (18). In comparison, our
268 approach of supplying foods and controlling for compliance and medication likely yields
269 higher precision in examining efficacy. Skoldstam *et al.* noted a decrease in CRP after a
270 Mediterranean diet compared to a control diet in patients with RA, but there was no effect on
271 ESR (19). However, the concurrent weight loss seen in the study by Skoldstam *et al*.
272 complicates the interpretation. The same research group has since published a follow-up
273 investigation based on pooled data, which indicate effects beyond weight reduction in
274 interventions with lacto-vegetarian, vegan or Mediterranean diets (20). Furthermore,
275 bDMARDs, powerful anti-inflammatory agents that were used by about a third of
276 participants in ADIRA, were uncommon when Skoldstam *et al.* carried out their study.

277 The chemokines CXCL1, CXCL5 and CXCL6, known for their chemoattractant
278 effects on neutrophils to the site of injury, infection or inflammation (21), decreased
279 significantly in the main analysis. In studies on synovial fibroblasts isolated from patients
280 with RA, CXCL1 is indicated to stimulate an inflammatory response and upregulate IL-6
281 expression (22). Previously, higher concentration of CXCL1 was reported in plasma and
282 synovial fluids from patients with RA compared to healthy volunteers, and CXCL1 is thus
283 suggested to play a mediating role in neutrophil recruitment into the inflamed joint (23).
284 Previous research also found increased CXCL1 expression linked to poor survival in cancer
285 (24). Further, increased circulating concentration of CXCL5 has been found in patients with
286 RA compared to healthy controls (25). Thus, lower circulating concentrations of CXCL1,

CXCL5 and CXCL6 as observed in the current study probably reflect reduced systemic inflammation.

TNFSF14 decreased significantly with the intervention diet compared to the control diet in the main analysis. Previously, higher concentration of TNFSF14 has been found in patients with RA in comparison to healthy controls (26). Furthermore, studies indicate a role of TNFSF14 as an osteoclast inducing protein promoting the progression of bone destruction in RA (26, 27).

The sensitivity analysis yielded similar significant effects in CXCL1 and CXCL6 as did the main analysis (**Supplemental Figure 1**). In addition, GDNF decreased significantly during the intervention diet period compared to the control diet period. As recently reviewed by Morel *et al*. (28), GDNF is produced by glial cells and binds primarily to GDNF family co-receptor α1 (GFRα1), expressed in a wide range of tissues. GDNF is described to have neuroprotective effects as well as regenerative effects on epithelial tissue upon infection or damage (28). Data on serum protein levels of GDNF in patients with RA are scarce, but one investigation has shown lower concentrations in plasma from patients with active RA compared to healthy controls (29). Thus, a lowered level of GDNF in serum could translate to decreased activation of inflammation-resolving pathways.

The ADIRA trial has several unique strengths. First, along with dietary advice, easily prepared foods were supplied to the participants' homes free of charge, which likely contributed to the high reported compliance. Second, we employed a crossover design to reduce the effects of inter-individual variation and maximize the statistical power from the available sample size. We also implemented a 4-month washout period, which we believe to be sufficient to normalize effects from the prior dietary period. Of most importance for

evaluating dietary effect, rather than effects related to energy balance, is that the participants were weight stable during the study.

This study also has some limitations. First, the generalizability can be questioned; those who completed both diet periods with high compliance with stable medication had higher educational level and a lower waist-to-hip ratio than the rest. The study design with provided food items may also be difficult to achieve in other populations, specifically in out-patient settings (i.e., patient compliance might be affected). Moreover, our study population was mainly highly educated, middle age or older females of European descent. Additionally, those who completed both diet periods with high compliance and stable medication had an even higher educational level as well as a lower waist-to-hip ratio than the rest of the participants. It is possible that effects from dietary manipulation may differ in younger, more diverse or less educated populations. Second, our investigation examined markers of inflammation in blood, and in serum isolated from blood, taken by venipuncture. As such, our results likely reflect systemic inflammation, or at least proteins exhibiting systemic effects. It is theoretically possible to examine local samples, such as for example synovial fluid, to explore the environment around the joints. However, due to procedural limitations and in consideration to participant comfort, we found it most suitable to collect blood samples. Third, our power-analysis and subsequent sample size was constructed to detect relevant effects on DAS28-ESR, not for analysis of biomarkers of inflammation. For this report, sample size was further reduced because the full set of serum samples was not used to quantify biomarkers. While we consider that our procedure of only analyzing correctly handled samples increases the reliability of our findings, the resulting lower sample size might have decreased the probability of detecting statistically significant differences. There is also a risk of bias; those with correctly handled samples did have lower leucocyte concentration as well as a slightly skewed macronutrient composition in their diet compared

to those not included. Finally, as the Olink panel analysis was performed without correction for multiple testing, we view the results presented here as an exploratory report on the potential effects of diet on markers of inflammation.

Conclusion

In conclusion, our results indicate that a Mediterranean-like diet intervention with proposed anti-inflammatory foods compared to a Western diet reduced the systemic inflammation in patients with RA that had a high compliance to the dietary intervention. These findings need to be interpreted carefully given the risk of type-1 error due to multiple hypothesis tests. The results warrant further studies to validate our findings and to evaluate the clinical relevance of changes in CXCL1, CXCL5, CXCL6, GDNF and TNFSF14 in patients with RA.

Acknowledgments

First and foremost we would like to express our gratitude to the participants who made this study possible. We want to acknowledge the work of the nurses Anneli Lund and Marie-Louise Andersson at the Clinical Rheumatology Research center at Sahlgrenska University Hospital, and the statistician David Bock at the Health Metrics Unit. We also would like to acknowledge the work from the home-food delivery chain mat.se.

Authors' contributions

AW, HML, LB and IG designed the study. EH, HML, LB, AW and ATW conducted the research. EH performed the statistical analysis, analyzed the data, wrote the first draft, and had primary responsibility for the final content. All authors helped interpreting the data and have read and approved the final manuscript. The authors declare no conflict of interest.

References

1. Hayter SM, Cook MC. Updated assessment of the prevalence, spectrum and case definition of autoimmune disease. Autoimmunity reviews 2012;11(10):754-65. doi: https://doi.org/10.1016/j.autrev.2012.02.001.
2. Minichiello E, Semerano L, Boissier M-C. Time trends in the incidence, prevalence, and severity of rheumatoid arthritis: A systematic literature review. Joint Bone Spine 2016;83(6):625-30. doi: https://doi.org/10.1016/j.jbspin.2016.07.007.
3. Yu C, Jin S, Wang Y, Jiang N, Wu C, Wang Q, Tian X, Li M, Zeng X. Remission rate and predictors of remission in patients with rheumatoid arthritis under treat-to-target strategy in real-world studies: a systematic review and meta-analysis. Clinical rheumatology 2019;38(3):727-38. doi: 10.1007/s10067-018-4340-7.
4. Palominos PE, Gasparin AA, de Andrade NPB, Xavier RM, da Silva Chakr RM, Igansi F, Gossec L. Fears and beliefs of people living with rheumatoid arthritis: a systematic literature review. Advances in Rheumatology 2018;58(1):1. doi: 10.1186/s42358-018-0001-4.
5. Salminen E, Heikkilä S, Poussa T, Lagström H, Saario R, Salminen S. Female Patients Tend to Alter Their Diet Following the Diagnosis of Rheumatoid Arthritis and Breast Cancer. Preventive Medicine 2002;34(5):529-35. doi: https://doi.org/10.1006/pmed.2002.1015.
6. Shivappa N, Steck SE, Hurley TG, Hussey JR, Hébert JR. Designing and developing a literature-derived, population-based dietary inflammatory index. Public health nutrition 2014;17(8):1689-96. doi: 10.1017/s1368980013002115.
7. Casas R, Sacanella E, Estruch R. The immune protective effect of the Mediterranean diet against chronic low-grade inflammatory diseases. Endocrine, metabolic & immune disorders drug targets 2014;14(4):245-54. doi: 10.2174/1871530314666140922153350.
8. Philippou E, Petersson SD, Rodomar C, Nikiphorou E. Rheumatoid arthritis and dietary interventions: systematic review of clinical trials. Nutrition Reviews 2020. doi: 10.1093/nutrit/nuaa033.
9. Vadell AKE, Bärebring L, Hulander E, Gjertsson I, Lindqvist HM, Winkvist A. Anti-inflammatory Diet In Rheumatoid Arthritis (ADIRA)—a randomized, controlled crossover trial indicating effects on disease activity. The American journal of clinical nutrition 2020;111(6):1203-13. doi: 10.1093/ajcn/nqaa019.
10. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham III CO, Birnbaum NS, Burmester GR, Bykerk VP, Cohen MD, et al. 2010 Rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis & Rheumatism 2010;62(9):2569-81. doi: 10.1002/art.27584.
11. Winkvist A, Barebring L, Gjertsson I, Ellegard L, Lindqvist HM. A randomized controlled cross-over trial investigating the effect of anti-inflammatory diet on disease activity and quality of life in rheumatoid arthritis: the Anti-inflammatory Diet In Rheumatoid Arthritis (ADIRA) study protocol. Nutrition journal 2018;17(1):44. doi: 10.1186/s12937-018-0354-x.
12. 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. Nutrition journal 2021;20(1):9. doi: 10.1186/s12937-021-00663-y.

13. Ekdahl C, Eberhardt K, Andersson SI, Svensson B. Assessing disability in patients with rheumatoid arthritis. Use of a Swedish version of the Stanford Health Assessment Questionnaire. Scandinavian journal of rheumatology 1988;17(4):263-71. doi: 10.3109/03009748809098795.
14. Assarsson E, Lundberg M, Holmquist G, Björkesten J, Thorsen SB, Ekman D, Eriksson A, Rennel Dickens E, Ohlsson S, Edfeldt G, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. PloS one 2014;9(4):e95192. doi: 10.1371/journal.pone.0095192.
15. Radner H, Chatzidionysiou K, Nikiphorou E, Gossec L, Hyrich KL, Zabalan C, van Eijk-Hustings Y, Williamson PR, Balanescu A, Burmester GR, et al. 2017 EULAR recommendations for a core data set to support observational research and clinical care in rheumatoid arthritis. Annals of the rheumatic diseases 2018;77(4):476-9. doi: 10.1136/annrheumdis-2017-212256.
16. Tishkowski K, Gupta V. Erythrocyte Sedimentation Rate. Edtion ed. StatPearls. Treasure Island (FL): StatPearls Publishing, Copyright © 2021, StatPearls Publishing LLC., 2021.
17. Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. The role of short-chain fatty acids in health and disease. Advances in immunology 2014;121:91-119. doi: 10.1016/b978-0-12-800100-4.00003-9.
18. McKellar G, Morrison E, McEntegart A, Hampson R, Tierney A, Mackle G, Scoular J, Scott JA, Capell HA. A pilot study of a Mediterranean-type diet intervention in female patients with rheumatoid arthritis living in areas of social deprivation in Glasgow. Annals of the rheumatic diseases 2007;66(9):1239-43. doi: 10.1136/ard.2006.065151.
19. Skoldstam L, Hagfors L, Johansson G. An experimental study of a Mediterranean diet intervention for patients with rheumatoid arthritis. Annals of the rheumatic diseases 2003;62(3):208-14.
20. Skoldstam L, Brudin L, Hagfors L, Johansson G. Weight reduction is not a major reason for improvement in rheumatoid arthritis from lacto-vegetarian, vegan or Mediterranean diets. Nutrition journal 2005;4:15. doi: 10.1186/1475-2891-4-15.
21. Luster AD. Chemokines--chemotactic cytokines that mediate inflammation. The New England journal of medicine 1998;338(7):436-45. doi: 10.1056/nejm199802123380706.
22. Hou S-M, Chen P-C, Lin C-M, Fang M-L, Chi M-C, Liu J-F. CXCL1 contributes to IL-6 expression in osteoarthritis and rheumatoid arthritis synovial fibroblasts by CXCR2, c-Raf, MAPK, and AP-1 pathway. Arthritis research & therapy 2020;22(1):251. doi: 10.1186/s13075-020-02331-8.
23. Koch AE, Kunkel SL, Shah MR, Hosaka S, Halloran MM, Haines GK, Burdick MD, Pope RM, Strieter RM. Growth-related gene product alpha. A chemotactic cytokine for neutrophils in rheumatoid arthritis. The Journal of Immunology 1995;155(7):3660.
24. Zhang Z, Chen Y, Jiang Y, Luo Y, Zhang H, Zhan Y. Prognostic and clinicopathological significance of CXCL1 in cancers: a systematic review and meta-analysis. Cancer biology & therapy 2019;20(11):1380-8. doi: 10.1080/15384047.2019.1647056.
25. Koch AE, Kunkel SL, Harlow LA, Mazarakis DD, Haines GK, Burdick MD, Pope RM, Walz A, Strieter RM. Epithelial neutrophil activating peptide-78: a novel chemotactic cytokine for neutrophils in arthritis. The Journal of clinical investigation 1994;94(3):1012-8. doi: 10.1172/JCI117414.
26. Edwards JR, Sun SG, Locklin R, Shipman CM, Adamopoulos IE, Athanasou NA, Sabokbar A. LIGHT (TNFSF14), a novel mediator of bone resorption, is elevated in

rheumatoid arthritis. Arthritis and rheumatism 2006;54(5):1451-62. doi: 10.1002/art.21821.

27. Ishida S, Yamane S, Nakano S, Yanagimoto T, Hanamoto Y, Maeda-Tanimura M, Toyosaki-Maeda T, Ishizaki J, Matsuo Y, Fukui N, et al. The interaction of monocytes with rheumatoid synovial cells is a key step in LIGHT-mediated inflammatory bone destruction. Immunology 2009;128(1 Suppl):e315-e24. doi: 10.1111/j.1365-2567.2008.02965.x.
28. Morel L, Domingues O, Zimmer J, Michel T. Revisiting the Role of Neurotrophic Factors in Inflammation. Cells 2020;9(4). doi: 10.3390/cells9040865.
29. Petersen LE, Baptista TSA, Molina JK, Motta JG, do Prado A, Piovesan DM, de Nardi T, Viola TW, Vieira ÉLM, Teixeira AL, et al. Cognitive impairment in rheumatoid arthritis: role of lymphocyte subsets, cytokines and neurotrophic factors. Clinical rheumatology 2018;37(5):1171-81. doi: 10.1007/s10067-018-3990-9.

Table 1. Baseline data of participants who completed at least one diet period without discontinued or new bDMARD or glucocorticoid treatment, grouped by inclusion in multiplex inflammation-related protein quantification

	Intervention-Control **n = 13**	**Control-Intervention** **n = 13**	**Not included** **n = 12**
Female	9 (69)	10 (77)	10 (83)
Age (year)	62 (55, 63)	66 (48, 72)	70 (61, 73)
Parental origin			
Europe	12 (92)	13 (100)	10 (83)
Africa	0 (0)	0 (0)	1 (8)
Asia	1 (8)	0 (0)	1 (8)
Non-smokers	11 (85)	13 (100)	12 (100)
Employment status			
Not employed	2 (15)	7 (54)	9 (75)
Employed < 15 hrs/wk	1 (8)	0 (0)	0 (0)
Employed 16-30 hrs/wk	3 (23)	1 (8)	0 (0)
Employed 31-40 hrs/wk	3 (23)	3 (23)	1 (7)
Employed > 40 hrs/wk	4 (31)	2 (15)	2 (17)
Educational level			
Junior high school	1 (8)	0 (0)	4 (33)
2 year senior high school	1 (8)	3 (23)	4 (33)
≥ 3 year senior high school	1 (8)	2 (15)	2 (17)
College or university	10 (77)	8 (62)	2 (17)
Medication usage			
bDMARD	4 (31)	5 (38)	6 (50)
csDMARD	10 (77)	9 (69)	9 (75)
No DMARD	2 (15)	2 (15)	1 (8)
Anthropometric measures			
BMI (kg/m^2)	27.1 (23.6, 32.8)	26.4 (24.2, 29.9)	27.7 (24.2, 33.5)
Waist-Hip ratio	0.84 (0.78, 0.98)	0.85 (0.83, 0.92)	0.82 (0.80, 0.88)

Laboratory data			
DAS28-ESR	3.9 (3.2, 4.7)	3.2 (2.9, 4.5)	3.6 (3.0 4.4)
HAQ	0.38 (0.13, 1.19)	0.38 (0.13, 1.31)	0.69 (0.31, 1.06)
CRP (mg/L)	2 (1, 4)	5 (1, 6)	3 (1, 5)
ESR (mm/h)	20 (13, 27)	14 (8, 26)	18 (10, 23)
WBC (10^9/L)	5.1 (4.3, 5.7)	6.3 (5.1, 7.6)	5.6 (4.8, 6.4)
Trombocytes (10^9/L)	250 (240, 310)	280 (250, 410)	240 (220, 280)
Dietary intake			
Energy (kcal/day)	1900 (1600, 2200)	1800 (1400, 2100)	1800 (1200, 2300)
Fat (E%)	38 (31, 42)	41 (36, 45)	35 (32, 37)
Saturated fatty acids (E%)	16 (14, 17)	15 (13, 16)	13 (11, 14)
Protein (E%)	16 (14, 18)	15 (14, 20)	15 (15, 22)
Carbohydrate (E%)	42 (39, 47)	38 (36, 42)	46 (39, 52)
Fiber (g/day)	19 (14, 22)	15 (13, 20)	19 (15, 21)
Vitamin-D (µg/day)	3 (2, 6)	5 (4, 10)	6 (5, 8)
Selenium (µg/day)	35 (32, 40)	48 (41, 75)	51 (42, 74)
Folate (µg/day)	260 (210, 330)	270 (220, 320)	220 (190, 280)

Values are median (25^{th} percentile, 75^{th} percentile) or n (%) unless otherwise indicated. bDMARD, Biological disease modifying anti-rheumatic drug; BMI, body mass index; csDMARD, Conventional synthetic disease modifying anti-rheumatic drug; DMARD, disease modifying anti-rheumatic drug; CRP, C-reactive protein; DAS28-ESR, Disease Activity Score-28 erythrocyte sedimentation rate; ESR, Erythrocyte sedimentation rate; HAQ, Health assessment questionnaire; WBC, White blood cell count.

Table 2. Modelled estimates of developments in clinically validated markers of inflammation within and between diet periods among patients with RA who did not discontinue or start any new disease modifying anti-rheumatic drug or glucocorticoid therapy[1]

	Intervention **Mean Change (95% CI)**	**Control** **Mean Change (95% CI)**	**Difference between diet periods[2]**	**95% CI**	**P**
Clinical markers of inflammation in those who completed at least one diet period regardless of compliance[3]					
CRP[4] (mg/L)	-0.042 (-0.167, 0.082)	0.09 (-0.034, 0.215)	-0.133	-0.304, 0.039	0.125
ESR (mm/h)	-0.709 (-3.485, 2.067)	3.071 (0.303, 5.838)	-3.779	-7.710, 0.152	0.059
Clinical markers of inflammation in those who completed both diet periods with high compliance[5]					
CRP[4] (mg/L)	-0.058 (-0.215, 0.100)	0.097 (-0.058, 0.251)	-0.154	-0.362, 0.054	0.136
ESR (mm/h)	-1.504 (-4.991, 1.982)	3.985 (0.566, 7.404)	**-5.490**	**-10.310, -0.669**	**0.027**

[1]Participants completing at least one diet period. CI, Confidence interval; CRP, C-reactive protein; ESR, Erythrocyte sedimentation rate; RA, Rheumatoid Arthritis.

[2]Intervention – Control, change during period values.

[3]Analyzed by use of a linear mixed model with period, treatment, body mass index and baseline value as fixed effects and subject as random effect, n = 38.

[4]To comply with model assumptions, log10-transformed values were used.

[5]Analyzed by use of a linear mixed model with period, treatment, body mass index and baseline value as fixed effects and subject as random effect, n = 29.

Figure legends

Figure 1. Flow chart of subject recruitment reported according to CONSORT. CRP and ESR was quantified in all participants' samples. Quantifying relative concentrations of inflammation-related proteins in serum samples in the multiplex assay was done only if samples had been handled according to the most strict protocol and in participants whose samples were available from all visits. Participants with new or discontinued DMARD or glucocorticoid treatment were excluded from analyses, and only those who completed both diet periods with high compliance were selected for a sensitivity analysis. CRP, C-reactive protein; DMARD, disease modifying anti-rheumatic drug, ESR, Erythrocyte sedimentation rate.

Figure 2. Changes in concentrations of inflammation-related proteins within and between dietary periods measured in participants completing at least one diet period who did not discontinue or start any new disease modifying anti-rheumatic drug or glucocorticoid therapy, n = 26. Black colored lines denotes $P < 0.05$. Concentrations are presented in an arbitrary, semi-quantitative log2 scale that is valid for comparison of relative concentrations between different time points within individuals, analyzed using a linear mixed model with period, treatment, BMI and baseline value as fixed effects and subject as random effect. See Supplemental Table 1 for abbreviations.

[1]Analyzed and presented on a log10 scale in order to comply with model assumptions.